ISSN: 2754-4761

Journal of Pulmonology Research & Reports

SCIENTIFIC Research and Community

Research Article

Open Access

COVID-19: Immunogenetics and Immuno-Epidemiological Parameters

Attapon Cheepsattayakorn[1]* and Ruangrong Cheepsattayakorn[2]

[1]10th Zonal Tuberculosis and Chest Disease Center, Chiang Mai, Thailand

[2]Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

ABSTRACT

As of February 15, 2020, 51,800 cases of COVID-19 disease, including more than 1,600 COVID-19-related deaths, had been laboratory-confirmed in mainland China, mainly in Hubei province. Additionally, 526 laboratory-confirmed cases have been reported across 25 other countries. Approximately, 15% of cases reported to the World Health Organization (WHO) are severe, 3% are critical, and 82% are mild clinical manifestations, whereas the estimated overall case fatality rate is approximately 2% but the figure outside of Hubei province is approximately 0.05% or less, not different from the fatality identified in the seasonal influenza. The mean age of COVID-19 patients is 52.4 years, whereas children and adolescents are the least likely group to be infected with the COVID-19, occurring in only 2 % of cases 19 years of age or younger. When the younger-age group get sick, they will get a mild form of COVID-19 without serious complications, with an average death rate of 0.2 %. Men constitute more than two-thirds of the reported COVID-19 cases (73 % vs. 27 %) and are more than 1.5 times more likely to die from COVID-19 (death rate : 2.8 % vs. 1.7 %). This sexual distinction of the anti-viral immunity between men and women is due to the genetic factors, hormonal factors, and environmental factors. The unanswered questions include the pathophysiology of pulmonary clinical infection, influenza and other viral co-infection, and the rate of bacterial complications. SARS-CoV-2 (COVID-19) infection has evolved to become a pandemic, in contrast to infections with SARS and MERS, whereas SARS-CoV-2 (COVID-19) has demonstrated having the similarities of genome sequence, receptor affinity, pathogenesis, and disease manifestation.

In conclusion, although genomic evidence does not support the belief that COVID-19 is a laboratory construct, currently it is impossible to disprove or prove the theories of its origin. To identify the COVID-19 origin, obtaining virus sequences from immediate non-human animal sources would be the most definite method. In the absence of proper cure of COVID-19, it is necessary to identify the factors that may assist in assessment of the COVID-19 disease severity before rapid progression of the disease.

***Corresponding author**

Attapon Cheepsattayakorn, 10th Zonal Tuberculosis and Chest Disease Center, 143 Sridornchai Road Changklan Muang Chiang Mai 50100 Thailand, Tel : 66 53 140767, 66 53 276364; Email : Attapon1958@gmail.com

Received: July 21, 2020; **Accepted:** July 27, 2020, **Published:** July 31, 2020

Keywords: COVID-19, SARS-Co-V-2, Immunogenetics, Immunopathology, Epidemiological Parameters, ABO Blood Type, Proximal Origin, Phylogenetic Analysis, Human Leukocyte Antigen

Abbreviations

ACE: Angiotensin-Converting Enzyme; ARDS: Acute Respiratory Distress Syndrome, BALF: Bronchoalveolar Lavage Fluid, CoV: Coronavirus; COVID-19: Coronavirus Disease 2019; 2019-nCoV: 2019-Novel Coronavirus; DAMPS: Damage-Associated Molecular Patterns, FCN: Ficolin, HLA: Human Leukocyte Antigen, ICU: Intensive Care Unit, IFITM1: Interferon-Induced Transmembrane Protein-1, IFN : Interferon, IL: Interleukin, ISGs: Interferon-Stimulated Genes, MERS: Middle-East Respiratory Syndrome; MRC: British Medical Research Centre; NK: Natural Killer, NKR: Natural Killer Receptor, OR: Odd Ratio, PD-1: Programmed Death Ligand-1, PAMPS: Pathogen-Associated Molecular Patterns, PBMCs: Peripheral Blood Monocytes, pDC: Plasmacytoid Dendritic Cells, RBD: Receptor Binding Domain; RNA: Ribonucleic Acid; SARS: Severe Acute Respiratory Syndrome; Tim-3: T-cell Immunoglobulin and Mucin-3, TNF: Tumor Necrosis Factor, WHO: World Health Organization

Introduction

As of February 15, 2020, 51,800 cases of COVID-19 disease, including more than 1,600 COVID-19-related deaths, had been laboratory-confirmed in mainland China, mainly in Hubei province [1]. Additionally, 526 laboratory-confirmed cases have been reported across 25 other countries [1]. Approximately, 15% of cases reported to the World Health Organization (WHO) are severe, 3% are critical, and 82% are mild clinical manifestations, whereas the estimated overall case fatality rate is approximately 2% but the figure outside of Hubei province is approximately 0.05% or less, not different from the fatality identified in the seasonal influenza [2, 3]. There is no expected cross protection by a common human coronavirus infection and theoretically, COVID-19 can infect any one of the individuals [3]. COVID-19 complications target especially the elderly, but the complications

can unpredictably occur in the younger age populations, as well as influenza [3]. Despite a low risk of COVID-19 complications, a mild clinical presentations of the disease allow a larger chain of transmission through various populations [3]. Critically, after first identified outbreak, understanding of epidemiology trajectory of infection still have a limitation. Although the WHO reported the dates of diagnosis of COVID-19 disease, but this is not enough information [3]. It is not yet clear why sustained chains of transmission have not been reported outside Asia. Genetic factors encouraging transmission within Asian populations, effective containment, inefficient transmission, poor reporting due to lacking molecular testing capacity in some low-income countries, and specific environmental conditions in Hubei province and mainland China [3].

Among the most severely affected patients, viral ribonucleic acid (RNA) has been detected in the plasma approximately 15% and viral detection in stool reveals possibility of fecal transmission [4, 5]. COVID-19 has been isolated in human saliva, nasopharynx and lower respiratory tract [6, 7]. Lacking lung biopsies or post-mortem sample investigations leads to an incomplete understanding of the pathogenesis of COVID-19 infection [3]. The innate immune cells are born capable of producing T regulatory cell cytokine (interleukin (IL)-10 and T helper 17 (TH17) cell cytokines (IL-6 and IL-23), but inability of induction of T helper 1 (TH1) cell cytokines (type I interferons (IFNs), IFN-γ, and IL-12) [8]. A new lineage of oligoclonal T cells that express natural killer (NK)-related receptors (NKR) is formed, whereas the diversity of the T-cell receptor (TCR) repertoire decreased with age [9]. In addition to the experimental evidence regarding the immunological features in neonates and children that are more prominent than in adults. In consideration of the pro-inflammatory response to the SARS-CoV infection, an overwhelming inflammatory reaction in aging population is a logical possibility [10]. The mean age of COVID-19 patients is 52.4 years, whereas children and adolescents are the least likely group to be infected with the COVID-19, occurring in only 2 % of cases 19 years of age or younger [11]. When the younger-age group get sick, they will get a mild form of COVID-19 without serious complications, with an average death rate of 0.2 % [12]. Men constitute more than two-thirds of the reported COVID-19 cases (73 % vs. 27 %) and are more than 1.5 times more likely to die from COVID-19 (death rate : 2.8 % vs. 1.7 %) [13]. This sexual distinction of the anti-viral immunity between men and women is due to the genetic factors, hormonal factors, and environmental factors. The unanswered questions include the pathophysiology of pulmonary clinical infection, influenza and other viral co-infection, and the rate of bacterial complications [3].

Human Leukocyte Antigen Map of COVID-19

HLA genotype plays a significant role in differential regulation and activation of T cells as well as disease duration and transmission [14]. A recent study on human leukocyte antigen (HLA) binding affinity of 48,395 unique peptides (possible 8- to 12-mers) from the SARs-CoV-2 (COVID-19) proteome for assessing the potential for cross-protective immunity conferred by previous exposures to common human coronaviruses (i.e. 229E, NL63, OC43, and HKU1) demonstrated that alleles HLA-A*02 : 02, HLA-B*15 : 03, and HLA-C*12 : 03 were the top presenters of conserved peptides. Fifty-six different HLA alleles, especially HLA-B*46: 01 revealed no appreciable binding affinity (<500 nm) to any of the conserved SARS-CoV-2 (COVID-19) peptides, indicating a concomitant lack of potential for cross-protective immunity from other human coronaviruses. Considering the entire proteome of SARS-CoV-2 (COVID-19), HLA-A and HLA-C alleles expressed the relative largest and smallest capacity to present SARS-CoV-2 (COVID-19) antigens, respectively. No appreciable global correlation between conservation of the SARS-CoV-2 (COVID-19) proteome and its predicted MHC binding affinity, indicating a lack of selective pressure for the capacity to present coronavirus epitopes ($p = 0.27$, Fisher's exact test). peptide presentation appears to be independent of estimated time of peptide production during SARS-CoV-2 (COVID-19) life cycle, with indistinguishable early and late SARS-CoV-2 (COVID-19) peptide presentation [14].

HLA binding assays carried out for 19 epitopes displaying positive T cell assays in all retrieved SARS-CoV-2 (C)OVID-19) proteins in a recent study demonstrated that five distinct alleles, HLA-A*02 : 01, HLA-B*40 : 01, HLA-DRA*01 : 01, HLA-DRB1*07 : 01, and HLA-DRB1*04 : 01 were positive. The population coverage of these alleles in China was 32.36 % and 59.76 % globally [15]. Previous studies demonstrated an increased severity towards the closely related SARS-CoV diseases in persons with HLA-B*46 : 01 [16] . Differences in HLA haplotype may influence the persons' response to SARS-CoV-2 (COVID-19) infection and some haplotypes may be associated with increased disease severity. Thus, HLA genotyping may help in identifying persons at risk. COVID-19 testing along with HLA genotyping is highly recommended to predict susceptibility to disease severity and assisting in future vaccination strategy plan.

Proximal Origin of Covid-19

COVID-19 (SARS-CoV-2) is the seventh member of the Coronaviridae known to infect humans [17]. Three of these viruses are: 1) SARS CoV-1, 2) MERS and 3) COVID-19 (SARS-CoV-2) can cause severe disease, and 4) HKU1, NL63, OC43 and 229E, are related to mild respiratory symptoms. The two notable characteristics of the COVID-19 genome are: 1) based on structural modelling and early biochemical experiments, COVID-19 is optimized for binding the human ACE2 receptor; and 2) the highly variable spike (S) protein of COVID-19 has a polybasic (furin) cleavage site at the S1 and S2 boundary via the insertion of twelve nucleotides. This event contributes to the acquisition of the three predicted O-linked glycans around the polybasic cleavage site [17].

Six residuals in the receptor binding domain (RBD) of the spike protein of SARS-CoV and SARS-related coronaviruses, the most variable part of the virus genome appear to be critical for binding to the human ACE2 receptor and the determining host range [18]. Five of these six residuals are mutated in COVID-19 compared to its most closely related virus, RaTG13 sampled from a *Rhinolophus affinis* bat to which it is approximately 96% identical [19]. COVID-19 seems to have an RBD that may bind with high affinity to ACE2 from human, non-human primate, cat, pig, and ferret [18]. COVID-19 may bind less efficiently to ACE2 in other species related to SARS-like viruses, such as rodents and civets [17]. Recent binding studies demonstrated that COVID-19 binds with high affinity to human ACE2 [20]. The COVID-19 spike appears to be the result of selection on human or human-like ACE2 permitting another suitable binding solution to occur and this strongly indicate that COVID-19 is not the genetic engineering product [17]. All COVID-19 sequenced genomes have the well adapted RBD and the polybasic cleavage site, and thus are derived from a common ancestor. Initial analyses demonstrated that Malayan pangolins (*Manis javanica*) illegally imported into Guangdong province, China contain a coronavirus (CoV) that is similar to COVID-19 [21, 22]. Nevertheless, no pangolin CoV has been identified to be sufficiently similar to COVID-19 across its entire genome for supporting direct human infection [17].

Phylogenetic Analysis of Covid-19

Volz., et al. demonstrated their study by analyzing 53 SARS-CoV-2 (COVID-19) whole genome sequences collected up to February 3, 2020. They found a strong association between the time of sample collection and accumulation of genetic diversity of COVID-19. By using Bayesian and maximum likelihood phylogenetic methods revealed that the COVID-19 was introduced into the human population in Wuhan, China in early December 2019 and has an epidemic doubling time of about 7 days. Precise estimated of epidemic size are not possible with current genetic data, the analyses demonstrated substantial heterogeneity in the number of secondary infections caused by each COVID-19-infected case that indicated by a high level of over-dispersion in the reproduction number [23]. Phylogenetic analysis demonstrates a common ancestor to SARS-CoV-2 (COVID-19), human SARS-CoV, and the bat SARS-CoV converge. The four structural viral proteins, envelope (E), membrane (M), nucleocapsid (N), and spike (S), imply a high degree of shared identity in range of 97.7-100 % between the SARS-CoV-2 (COVID-19) and bat coronaviruses that supports the descend of SARS-CoV-2 (VID-19) from an animal [24].

ABO Blood Type Associated Covid-19

A recent study conducted by Zhao et al on the correlation compared between the ABO blood group among 1,775 COVID-19-infected patients and 3,694 normal individuals from Wuhan city, China and 23,386 normal persons from Shenzhen city, China revealed that blood group A had a specific significant higher risk for COVID-19 compared to non-blood group A groups (albeit modest effect size, odd ratio (OR) = 1.20, $p = 0.02$), while blood group O had a significant lower risk for COVID-19 compared to non-O blood groups (OR = 0.67, $p < 0.001$) [25].

Tanigawa et al compared blood group O frequencies between the Shenzhen controls and the UK biobank Chinese group and demonstrated that the study results were consistent with the study results conducted by Zhao et al. Nevertheless, Tanigawa et al found that the frequency of blood group O was different between the Wuhan controls and the UK Biobank Chinese group (p = 0.00121) indicating careful consideration of inferences regarding ABO blood group differences [26].

COVID-19 Induced Inflammation

Cytokine and chemokines play a significant role in anti-viral immunity. In COVID-19, the primary cause of acute respiratory distress syndrome (ARDS) and multiple organ failure is related to cytokine storm, a phenomenon of excessive inflammatory reaction mediated by the rapid production of large amounts of cytokines in response to infection [27, 28]. Initial delay in cytokine and chemokine secretion by innate immune cells with subsequent surge in pro-inflammatory cytokines and chemokines (CCL2, CCL5, IFNs, IL-1β, IL-6, IL-8, MCP-1) by the activated macrophages and other recruited lymphocytes is observed in COVID-19, contributing to the recruitment and activation of adaptive immune cells (neutrophils, NK cells, and T cells) along with further production of pro-inflammatory cytokines, and finally causing a cytokine storm and tissue damage [28]. In COVID-19 patients, elevated serum cytokines are G-CSF, GM-CSF, IFN-γ, IL-1β, IL-2, IL-7, IL-8, IL-9, IL-10, IL-17, IP10, MCP-1, MIP-1A, MIP-1B, and TNF-α [29]. Particularly, IL-1, IL-6, and Tumor Necrosis Factor (TNF)-α cytokines secreted by macrophages are significantly higher in severe patients compared to non-severe cases [29]. IL-6, a multifunctional cytokine involving the formation of follicular helper T cells, generation of plasma cells, and differentiation of Th 17 cell subsets plays a primary role in cytokine storm that occurs in patients with COVID-19 [30]. IL-6 also inhibit IFN-α, therefore, suppress CD8+ cytotoxic T cells [30]. As demonstrated by *PD-1* and *Tim-3* expressions, IL-6 induces T cell exhaustion, thus, T cell-mediated immune response might be suppressed during cytokine storm [31]. Role of IL-6 in COVID-19 disease severity has been demonstrated by increased IL-6 levels and its positive association with disease severity [31-38]. A recent report from Germany revealed that COVID-19 cases with IL-6 levels of at least 80 pg/ml had a 22-fold increased risk of respiratory failure with median time to mechanical ventilation of 1.5 days [39] and higher serum IL-6 levels were also reported even 24 hours before death [39-41]. Thus, IL-6 could be used for early detection of COVID-19 patients at risk for respiratory failure as a single parameter or in association with other parameters.

TNF-α, a pro-inflammatory or a pro-apoptotic cytokine may contribute to apoptosis of aged T cells that express high TNFR1 (receptor of TNF-α) [42]. Few previous studies among aging patients (> 60 years) revealed that TNF-α level were significantly higher along with decreased T cell counts and increased levels of *PD1* and *Tim-3* (T cell exhaustion markers). This study results indicate the role of TNF-α as a negative regulator of T cell proliferation or survival [31]. Nevertheless, few previous studies on COVID-19 reported a negative correlation of TNF-α with T cell count and reported no difference in TNF-α levels in patients with COVID-19 [29, 43-46]. In ARDS, IL-1β and its family (IL-18, IL-33) are significant players to increase the recruitment of immune cells subsequent production of cytokines [47]. IL-1β and TNF-α are required to develop Th17 cells and assist in Th17 mediate immune response and increased vascular permeability [47]. IL-17 and GM-CSF, cytokines of the Th17 pathway increasing in patients with severe COVID-19 have researchers urgently investigate the role of Th17 in severe COVID-19 cases [48, 49]. Th17 cell-increased expression in the peripheral blood of patients with COVID-19 indicates a player in the COVID-19 cytokine storm as reported in the patients with MERS and SARS [50]. Some Th17 pathway-specific cytokines, such as GM-CSF, IL-1β, IL-17, and TNF-α are elevated in severe COVID-19 patients A case study on severe COVID-19 demonstrated an elevated count of Th17 cells, activated CD4+, and CD8+ T cells, whereas another previous study revealed a decrease in Th17 subset indicated by low IL-17 secretion urges the need to investigate the role of Th17 specific response in COVID-19 [48, 51 & 52]. An increased IFN-γ, IL-1β, IP-10, and MCP-1 serum concentrations contribute to the activation of Th1 cell response and further aggravation the cytokine storm like the occurrence in MERS-CoV and SARS-CoV [53, 54]. A previous study on three COVID-19 patients demonstrated that IL-1 expressed significant expression changes prior to deterioration of the respiratory function, whereas the other pro-inflammatory cytokines were induced only after occurrence of respiratory symptoms. These IL-1 expression changes indicate the role of the IL-1 pathway in the initial progression of COVID-19-associated pulmonary immunopathology and the IL-1 receptor signaling in respiratory epithelium-inflammatory damage [55]. An increase in anti-inflammatory cytokines of Th2 cells (IL-4 and IL-10) were identified in SARS-CoV-2 (COVID-19) patients, in contrast to SARS without clarification [56].

In severely ill-COVID-19 patients (approximately 15 % of patients with COVID-19), there were significant elevated levels of serum G-CSF, IL-2, IL-7, IL-10, IL-17, MCP-1, MIP-1A, and TNF-α in comparison to non-severely ill cases, indicated diverse cytokine profile in the two groups and cytokine storm in disease progression and severity involvement [29]. Cytokine storm accompanies the transition from mild to severe form of

COVID-19. A recent study on immunophenotype the anti-viral response in 4 COVID-19 patients by using single-cell transcriptome sequencing of peripheral blood monocytes (PBMCs), collected at the periods of pre-Intensive Care Unit (ICU) stay, ICU stay, and post-ICU stay demonstrated that there was a significant increase in monocytes and plasmacytoid dendritic cell populations in the ICU-stay specimens compared to the pre- and post-ICU-stay samples [57]. A reported gene signature in the ICU specimens demonstrated elevation of expression of interferon-stimulated genes (ISGs), such as *IFITM1* when compared to pre- and post-ICU specimens, indicating a significant viral load regulated type I interferon response (Type-1 IFN response) in onset of ARDS and disease progression [57]. Thus, there is evidence of dampened or delayed type interferon response in the initial phase of COVID-19 infection with subsequent increase in active viral replication that is a part of SARS-CoV pathogenesis [58, 59].

Dysregulation of Immune Cell Subset

COVID-19 viral replication induced pyroptosis contributes to release of Damage-Associated Molecular Pattern (DAMPS), IL-1β, and Pathogen-Associated Molecular Pattern (PAMPS), such as ATP, ASC oligomers, and viral nucleic acid that induce the production of pro-inflammatory cytokines and chemokines, such as IL-6, IL-10, MCP-1, MIP-1α, MIP-1β from alveolar macrophages and adjacent epithelial cells [29, 54]. Therefore, the resultant microenvironment attracts both innate and adaptive immunity cells and pro-inflammatory cascade sets [29, 54]. Pulmonary macrophages are the primary players in both uncontrolled immunopathology of COVID-19 and effective host immunity against COVID-19 that contribute to cytokine storm by secretion of IP-10, MCP-1, MCP-1α, etc. [60]. A recent study indicated that major host immune dysregulations include recruitment of pro-inflammatory cells, such as monocytes and neutrophils, viral load-induced hyperinflammation, and dampened type-1 IFN response [59]. Type-1 IFN response is critical for induction of effectively adaptive response and controlling viral replication.

Single cell RNA-sequencing-based characterization of bronchoalveolar lavage fluid (BALF) from three severely ill, three mild COVID-19 patients and eight healthy subjects demonstrated that a monocyte-derived *FCN-1*$^{+}$ macrophages were the predominant macrophage in the BALF [61]. An elevated level of CD14^{+} and CD16^{+} monocyte subset was revealed in patients with COVID-19 when compared to healthy subjects and also demonstrated higher level in COVID-19 patients requiring ICU admission [62]. A previous study on immunophenotyping the antiviral response in 4 patients with COVID-19 (male young, male elderly, female young, female elderly) by using PBMCs collected pre-ICU, during ICU, and post-ICU stays demonstrated a significant increase in monocytes and plasmacytoid dendritic cells (pDC) populations and a gene signature of elevated expression of DDX58, IRF8, TLR7, and ISGs like *IFITM1* in the ICU patients' specimens, when compared to pre- and post-ICU specimens [52]. Therefore, there is evidence of delayed or dampened type-1 IFN response in the initial phases of the infection with subsequent increase with active viral replication, a part of pathogenesis of SARS-CoV [50, 59]. A previously subsequent study in 50 COVID-19 patients of varying severity involving profiling of cytokine levels, whole blood transcriptome, and immune cells demonstrated a significant impaired type-1 IFN response in the critical patients, characterized by reduced levels of IFN-α and IFN-β accompanying with high levels of IL-6 and TNF-α. This study also revealed a significant downregulation of 6 ISGs that specify type-1 IFN response in severe COVID-19 patients and reduction of pDC population compared to healthy subjects [63].

Conclusion

SARS-CoV-2 (COVID-19) infection has evolved to become a pandemic, in contrast to infections with SARS and MERS, whereas SARS-CoV-2 (COVID-19) has demonstrated having the similarities of genome sequence, receptor affinity, pathogenesis, and disease manifestation. Although genomic evidence does not support the belief that COVID-19 is a laboratory construct, currently it is impossible to disprove or prove the theories of its origin. To identify the COVID-19 origin, obtaining virus sequences from immediate non-human animal sources would be the most definite method. Additionally, experimental studies of the role of the polybasic cleavage site and predicted O-linked glycans and receptor binding would be more helpful to obtain more viral genetic data. For substantially refining of phylogenetic estimates of epidemic size and growth rate of COVID-19 in Wuhan, Hubei province and mainland China, larger numbers of more systematically sampled sequences from across China are needed. Only a small proportion of COVID-19-infected patients progress to severe COVID-19 requiring critical care. In the absence of proper cure of COVID-19, it is necessary to identify the factors that may assist in assessment of the COVID-19 disease severity before rapid progression of the disease. Nevertheless, our knowledge of SARS or MERS has not enough to restrain current COVID-19 pandemic.

References

1. World Health Organization (2020) Coronavirus 2019 (COVID-19): situation report-26, February 15. Geneva: World Health Organization https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports.
2. WHO press conference (2020) https://www.who.int/emergencies/diseases/novel-coronavirus-2019/media-resources/press-briefings.
3. Vetter P, Isabella Eckerle, Laurent Kaiser (2020) Covid-19: a puzzle with many missing pieces, with better information on epidemiology, pathogenesis, and treatments are urgent priorities. British Medical Journal 368: 627.
4. Huang C, Yeming Wang, Xingwang Li, Lili Ren, Jianping Zhao, et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan. 395: 497-506.
5. Zhang H, Zijian Kang, Gong Haiyi, Da Xu, Jing Wang, et al. (2020) The digestive system is a potential route of 2019-nCoV infection: a bioinformatics analysis based on single-cell transcryptomes. BioRxiv.
6. KK, Owen Tak-Yin Tsang, Cyril Chik-Yan Yip, Kwok-Hung Chan, Jacky M C Chan, et al. (2020) Consistent detection of 2019 novel coronavirus in saliva. Clinical Infectious Diseases.
7. Zhu N, Dingyu Zhang, Wenling Wang, Xingwang Li, Bo Yang, et al. (2020) China novel coronavirus investigating and research team. A novel coronavirus from patients with pneumonia in China, 2019. New England Journal of Medicine v- 382: 727-733
8. Prendergast AJ, Klenerman P, Goulder PJR (2012) The impact of differential antiviral immunity in children and adults. Nat Rev Immunol 12: 636-648.
9. Vallejo AN (2006) Age-dependent alterations of the T cell repertoire and functional diversity of T cells of the aged. Immunol Res 36: 221-228.
10. Totura AL, Baric RS (2012) SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr Opin Virol 2: 264-275.
11. Qian K, Deng Y, Tai Y, Jun Peng, Hao Peng, et al. (2020) Clinical characteristics of 2019 novel infected coronavirus pneumonia : a systematic review and meta-analysis. medRxiv.
12. Wei M, Yuan J, Liu Y, Tao Fu, Xue Yu, et al. (2020) Novel

coronavirus infection in hospitalized infants under 1 year of age in China. JAMA 323: 1313-1314.
13. Klein SL, Flanagan KL (2016) Sex differences in immune responses. Nat Rev Immunol 16: 626-638.
14. Nguyen A, David JK, Maden SK, Wood MA, Weeder BR, et al. (2020) Human leukocyte antigen susceptibility map for SARS-COV-2. Journal of Virology.
15. Sanchez-Mazas A (2020). HLA studies in the context of coronavirus outbreaks. Swiss Medical Weekly 150 : w20248.
16. Stephens HAF, Klaythong R, Sirikong M, Vaughn DW, Green S, et al. (2002) HLA-A and -B allele associations with secondary dengue virus infections correlate with disease severity and the infecting viral serotype in ethnic Thais. Tissue Antigens 60: 309-318.
17. Andersen KG (2020) The proximal origin of SARS-CoV-2. Arambaut ARTIC Network 26: 450-452.
18. Wan Y (2020) Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. Journal of Virology.
19. Wu F (2020) A new coronavirus associated with human respiratory disease in China. Nature 579: 265-269.
20. Wrapp D (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. bioRxiv.
21. Liu P (2019) Viral metagenomics revealed sendai virus and coronavirus infection of Malayan pangolins (Manis javanica). Viruses 11: 979.
22. Wong MC, Sara J Javornik Cregeen (2020) Evidence of recombination in coronaviruses implicating pangolin origins of nCoV-2019. bioRxiv.
23. Volz E (2020) Adult Latif Jameel Institute for Disease and Emergency Analytics (J-IDEA), and Imperial College London. Report WHO Collaborating Centre for Infectious Disease Modelling, MRC Centre for Global Infectious Disease Analysis 5: Phylogenetic analysis of SARS-CoV 2: 7.
24. Ramaiah A, Arumugaswami V (2020) Insights into cross-species evolution of novel human coronavirus 2019-nCoV and defining immune determinants for vaccine development. bioRxiv.
25. Zhao J, Yang Y, Huang H-P, Li D, Gu D-F, et al. (2020) Relationship between the ABO blood group and the COVID-19 susceptibility. MedRxiv.
26. Tanigawa Y, Rivas MA (2020) Initial review and analysis of COVID-19 host genetics and associated phenotypes. Preprints 2020.
27. Chousterman BG, Swirski FK, Weber GF (2017) Cytokine storm and sepsis disease pathogenesis. Semin Immunopathol 39: 517-528.
28. Ye Q, Wang B, Mao J. (2020) The pathogenesis and treatment of the " Cytokine Storm " in COVID-19. J Infect 80: 607-613.
29. Huang C, Wang Y, Li X, Ren L, Zhao J, et al. (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The Lancet 395: 497-506.
30. Velazquez-Salinas L, Verdugo-Rodriguez A, Rodriguez LL, Borca MV (2019) The role of interleukin 6 during viral infections. Front Microbiology 10: 1057.
31. Diao B, Wang C, Tan Y, Chen X, Liu Y, et al. (2020) Reduction and functional exhaustion of T cells in patients with coronavirus disease 2019 (COVID-19). medrxiv.
32. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. (2020) Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19)-infected patients. British Journal of Haematology 189: 428-437.
33. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, et al. (2020) Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2-infected patients. medrxiv.
34. Liu T, Zhang J, Yang Y, Ma H, Li Z, et al. (2020) The potential role of IL-6 in monitoring severe case of coronavirus disease 2019. Medrxiv.
35. Wu C, Chen X, Cai Y, Xia J, Zhou X, et al. (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus diseases 2019 pneumonia in Wuhan, China. JAMA Internal Medicine.
36. Chen X, Zhao B, Qu Y, Chen Y, Xiong J, Feng Y, et al. (2020) Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely associated with drastically elevated interleukin-6 (IL-6) level in critically ill COVID-19 patients. Infectious Diseases.
37. Liu F, Li L, Xu M, Wu J, Luo D, et al. (2020) Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. Journal of Clinical Virology 127: 104370.
38. Zhu Z, Cai T, Fan L, Lou K, Hua X, Huang Z, et al. (2020) Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. International Journal of Infectious Disease 95: 332-339.
39. Herold T, Jurinovic V, Arnreich C, Hellmuth JC, von Bergwelt-Baildon M,et al. (2020) Level of IL-6 predicts respiratory failure in hospitalized symptomatic COVID-19 patients Infectious Diseases https://medrxiv.org/lookup/doi /10.1101/2020.04.01.20047381.
40. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. (2020) Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China : a retrospective cohort study. Lancet 395: 1054-1062.
41. Zhang B, Zhou X, Qiu Y, Feng F, Feng J, et al. (2020)Clinical characteristics of 82 death cases with COVID-19 https:// medrxiv.org/lookup/doi/10.1101/2020.02.26.20028191.
42. Gupta S, Bi R, Kim C, Chiplunkar S, Yel L, et al. (2005) Role of NF-κB signaling pathway in increased tumor necrosis factor-α-induced apoptosis of lymphocytes in aged humans. Cell Death Differ 12: 177-183.
43. He R, Lu Z, Zhang L, Fan T, Xiong R, et al. (2020) The clinical course and its correlated immune status in COVID-19pneumonia. Journal of Clinical Virology 127: 104361.
44. Chen G, Wu D, Guo W, Cao Y, Huang D, Wang H, et al. (2020) Clinical and immunological features of severe and moderate coronavirus disease 2019. Journal of Clinical Investigation 130: 2620-2629.
45. Ouyang Y, Yin J, Wang W, Shi H, Shi Y, Xu B, et al. (2020) Down-regulated gene expression spectrum and immune responses changed during the disease progression in COVID-19 patients. Clinical Infectious Diseases ciaa462 clin infect dis.
46. Li Y, Hu Y, Yu J, Ma T (2020) Retrospective analysis of laboratory testing in 54 patients with severe- or critical-type 2019 novel coronavirus pneumonia. Laboratory Investigation 100: 794-800.
47. Tisoncik JR, Korth MJ, Simmons CP, Farrar J, Martin TR, et al. (2012) Into the eye of the cytokine storm. Microbiology and Molecular Biology Review 76: 16-32.
48. Wu D, Yang XO (2020) Th17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor Fedratinib. J Microbiol Immunol Infect 53: 368-370.
49. Yang X, Dai T, Zhou X, Qian H, Guo R, et al. (2020) Analysis of adaptive immune cell populations and phenotypes in the patients infected with SARS-CoV-2. Infectious Diseases.https:// medrxiv.org/lookup/doi/10.1101/2020.03.23.20040675 .

50. Channappanavar R, Perlman S (2017) Pathogenic human coronavirus infections : causes and consequences of cytokine storm and immunopathology. Semin Imunopathol 39: 529-539.
51. Xu Z, Shi L, Wang Y, Zhang J, Huang L,et al. (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respiratory Medicine. https://linkinghub.elsevier.com/retrieve/pii/S221326002030076X .
52. Giamarellos-Bourboulis EJ, Netea MG, Rovina N, Akinosoglou K, Antoniadou A, et al. (2020) Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe 27: 992-1000.
53. Mahallawi WH, Khabour OF, Zhang Q, Makhdoum HM, Suliman BA (2018) MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17 cytokine profile. Cytokine 104: 8-13.
54. Wong CK, Lam CWK, Wu AKL, Ip WK, Lee NLS, et al. (2004) Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. Clinical and Experimental Immunology 136: 95-103.
55. Ong EZ, Chan YFZ, Leong WY, Lee NMY, Kalimuddin S, et al. (2020) A dynamic immune response shapes COVID-19 progression. Cell Host Microbe v-27.
56. Chien J-Y, Hsueh P-R, Chen W-C, Yu C-J, Yang P-C (2006) Temporal changes in cytokine/chemokine profiles and pulmonary involvement in severe acute respiratory syndrome. Respirology 11: 715-722.
57. Wei L, Ming S, Zou B, Wu Y, Hong Z, Li Z, et al. (2020) Viral invasion and type I interferon response characterize the immunophenotypes during COVID-19 infection. SSRN Electron J.
58. Channappanavar R, Fehr AR, Vijay R, Mack M, Zhao J, et al. (2016) Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microbe 19: 181-193.
59. Prompetchara E, Ketloy C, Palaga T (2020) Immune responses in COVID-19 and potential vaccines : lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol 3: 1-9.
60. Tay MZ, Poh CM, Renia L, MacAry PA, Ng LFP (2020) The trinity of COVID-19 : immunity, inflammation, and intervention. Nat Rev Immunol 20: 363-374.
61. Liao M, Liu Y, Yuan J, Wen Y, Xu G, et al. (2020) The landscape of lung bronchoalveolar immune cells in COVID-19 revealed by single-cell RNA sequencing. https://medrxiv.org/lookup/doi/10.1101/2020.0223.20026690 .
62. Zhou Y, Fu B, Zheng X, Wang D, Zhao C, et al. (2020) [Pathogenic T cells and inflammatory monocytes incite inflammatory storm in severe COVI-19 patients. Natl Sci Rev 7: 998-1002.
63. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Pere H, et al (2020) Impaired type-1 interferon activity and exacerbated inflammatory responses in severe COVID-19 patients. https://medrxiv.org/lookup/doi/10.1101/2020.0419.20068015.