Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Short Communication

SARS-CoV-2-induced immunodysregulation and the need for higher clinical suspicion for co-infection and secondary infection in COVID-19 patients

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Abstract Cases of co-infection and secondary infection emerging during the current Coronavirus Disease-19 (COVID-19) pandemic are a major public health concern. Such cases may result from immunodysregulation induced by the SARS-CoV-2 virus. Pandemic preparedness must include identification of disease natural history and common secondary infections to implement clinical solutions.

The COVID-19 pandemic, caused by the SARS-CoV-2 virus, is a major public health concern with over 27 million infections and almost 900 thousand fatalities worldwide reported as of September 9th, 2020.[1] Recent literature determined that the SARS-CoV-2 infection is associated with immune dysfunction.[2] Research on patients diagnosed with COVID-19, identified lymphopenia with decreased lymphocyte function, and CD4+, CD8+ T cell numbers,[2—4] B cells, and natural killer (NK) cells.[5] Furthermore, cell markers of immunosuppression[6] and T-cell exhaustion[2,3] are found in patients with COVID-19. Documented cases are emerging with secondary bacterial, viral and fungal infections.[4,7—9] Currently, the pathogenic mechanisms underlying the SARS-CoV-2 infection associated with immune dysfunction that permits secondary infection are not well understood. We speculate that the immunopathogenesis of the SARS-CoV-2 infection increases susceptibility of co-infection in COVID-19 patients. Pandemic preparedness must include the identification of possible co-pathogens, the implementation of enhanced diagnostic guidelines, and the development of

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therapeutic protocols in order to reduce COVID-19 morbidity and mortality. This article discusses the role of lymphopenia in COVID-19 co-infection and is supported by current literature, including reports of co-infection and studies on immunopathogenesis of lymphopenia.

Co-infection prevalence is estimated to be as high as 50% among COVID-19 fatalities.[9] Factors such as illness severity and common pathogens must be considered to ensure co-infection detection and treatment. A retrospective cohort study demonstrated a positive association between COVID-19 illness severity and co-infection using a multivariable regression analysis ($F = 10.507$, $R = 0.257$, P-value $= 0.014$). Within the 354 patients, co-infection was diagnosed in 23.5% and 24.4% of severe and critical cases, respectively. Suspected co-infection diagnostic testing in 116 out of 354 hospitalized COVID-19 patients identified bacterial, fungal and viral co-pathogens: *Acinetobacter baumannii, Escherichia coli, Candida albicans, Staphylococcus haemolyticus,* Mycoplasma *pneumonia, Pseudomonas aeruginosa, Stenotrophomonas maltophilia, Enterococcus faecium, Candida tropicalis, Candida parapsilosis, Candida lusitaniae* and Boca virus. Co-infection was identified in 66.64% (7/11) of the COVID-19 fatalities observed in the study. The critical patient group exhibited the highest mortality, rate of co-infection and lowest lymphocyte counts. Identification of these pathogens involved bronchoalveolar lavage, sputum and blood culture.[7] Co-infections in two critical COVID-19 patients include *A. baumannii* and *S. haemolyticus* infection in one case, and *E. coli* and *C. tropicalis* in another case. *C. albicans* and drug resistant *A. baumannii* co-infections were only identified in COVID-19 patients with critical illness.[7] Additionally, a COVID-19 case series presented a co-infection rate of >19% (4/21) with two patients with influenza A, one with parainfluenza type 3, and one with bacteremia from the opportunistic pathogen *P. aeruginosa*.[8] A literature review of COVID-19 identified co-infection cases with the bacterial, fungal and viral co-pathogens: *C. albicans*, *Candida glabrata*, *M. pneumoniae*, *Legionella pneumophila*, Panton- Valentine leukocidin-secreting *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Chlamydophila pneumoniae*, *Klebsiella pneumoniae*, *A. baumannii*, *Aspergillus flavus*, rhinovirus, enterovirus, parainfluenza, metapneumovirus, respiratory syncytial virus, adenovirus, influenza B virus, and influenza A virus. Influenza A virus was the most common co-pathogen.[9] False-negative RT-PCRs for SARS-CoV-2 were found in two patients infected with influenza A virus. Diagnosing pulmonary pathogens or co-infection based on false-negative results for SARS-CoV-2 poses a significant risk to patients and may hinder appropriate treatment in the upcoming influenza season.[9] A retrospective study identified 3 out of 11 (27.3%) patients with severe illness to have bacterial and fungal secondary infections, compared to zero secondary infections in patients with moderate illness.[4] More co-infection and secondary infection cases are expected to be reported. Identification of co-infection prevalence, and associated co-pathogens are paramount in targeted prevention, prophylaxis, testing and therapy.

Numerous studies demonstrate COVID-19 associated lymphopenia and immune system dysregulation particularly affecting T cells.[2,5,10] Due to the T cell role in humoral immunity, specifically the activation of B cell antibody production, decline in CD4 T cell or T helper cell subsets may impact immunocompetency.[10] A cohort study of 522 COVID-19 positive patients determined "counts of total T cells, CD8 or CD4 T cells lower than 800, 300, or 400/μL, respectively, were negatively correlated with patient survival".[2] Patients responsive to treatment exhibit increases in CD8 T cells and B cells; however, no changes in these cell subsets are observed in patients clinically unresponsive to treatment.[5] A case—control study of peripheral blood flow-cytometry analysis demonstrated a decrease in total lymphocytes, CD4, CD8, B cells, and natural killer (NK) cells in COVID-19 patients in comparison to healthy controls. In addition, an association between increased COVID-19 illness severity and decreased lymphocytes was found.[5] Additional studies support this finding, as increased COVID-19 severity is associated with decreased absolute numbers of CD4, CD8,[6] CD3 T cells, and B cell populations.[4,9] One study demonstrated that survival and proliferation of T cells, including CD4 and CD8 T cell subsets, are correlated with decreased serum levels of immunomodulators such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-10 in COVID-19 patients.[2] Additional studies found IL-2R, IL-6, and IL-10 to be increased in severely ill patients.[3,4] The proinflammatory molecule, TNF-α was higher in patients that experienced severe illness in comparison to patients that experienced mild or moderate illness.[2,4] However, in patients that experienced critical illness, TNF-α was significantly lower in comparison to patients that experienced mild or severe illness.[7] Association of interferon gamma (IFN-γ) producing T cells and illness yielded mixed results. For example, a study demonstrated a positive correlation between illness severity and the percentage of IFN-γ producing T cells,[3] while another study found that IFN-γ producing T cells were lower in patients with severe COVID-19 illness in comparison to patients with moderate COVID-19 illness.[4] T cell activation markers, HLA-DR and CD45RO were higher in patients with severe illness in comparison to those with mild COVID-19 illness. Additionally, the co-stimulatory marker, CD28, was lower in severely ill patients.[3] B cell and dendritic cell activation were decreased in patients with extremely severe COVID-19 illness.[3] T-cell exhaustion was signalled by increased expression of cell surface markers, programmed cell death receptor-1 (PD-1), and T cell immunoglobulin mucin-3 (Tim-3) during prolonged inflammatory response in later stages of COVID-19 illness.[2,3] Researchers speculate that prolonged illness is associated with lymphocyte apoptosis and anergy.[3] An observational study compared circulating CD14+ monocyte human leukocyte antigen-DR (mHLA-DR) expression between 16 COVID-19 positive patients categorized as critically ill or non-critically ill. Down-regulation of mHLA-DR is an indicator of immunosuppression and was determined to be significantly lower in critically ill patients in comparison to non-critically ill patients.[6] These researchers suggested implementation of an "immune-monitoring program" for critically ill patients to identify those that may benefit from immunological treatment.[6] These studies demonstrate the negative consequences of the SARS-CoV-2 virus on the immune system and suggest that COVID-19 infection, particularly severe infection, is associated with CD4 T-helper cell depletion, permits co-infection, or superinfection.

Table 1 Co-infection prevalence among COVID-19 patients.

Title	Country	Study Type	Participants	Co-infection Prevalence
Clinical characteristics and co-infections of 354 hospitalized patients with COVID-19 in Wuhan, China: a retrospective cohort study.	China	Observational	354 inpatients	3 of 76 (3.95%) confirmed in patients with suspected respiratory viral pathogens co-infection. 20 of 40 (50%) confirmed in patients with suspected bacterial and fungi co-infection. 7 of 11 (66.64%) confirmed co-infections in COVID-19 fatalities.
Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington state.	United States	Observational	21 inpatients	4 of 21 (19.05%) patients confirmed with bacterial and viral co-infection.
Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents?	Taiwan	Review	Not Available	Estimated rate of co-infection may be as high as 50% among COVID-19 non-survivors.

The current study identified a variable co-infection prevalence of 19.04%—66.64% among severe, critical, and non-survivor COVID-19 patients (Table 1). Studies included in this review determined co-infection with bronchoalveolar lavage, sputum and/or blood culture; however, these diagnostic methods were not specified within each study and are likely inconsistent across patients. For example, Lv et al. (2020) only tested for co-infection in 116/354 cases, while other studies did not specify testing rates. It is possible that specific co-pathogens were undetected without the use of broad diagnostic testing. As such, prevalence of co-infection may be higher and include under or undetected co-pathogens. In addition, the validity of implemented diagnostic methods may be impacted by timing of antimicrobial administration, false-negatives, detection of normal flora, or medical error. In particular, clinicians should proceed with particular suspicion of influenza A co-infection and the possibility of false-negative SARS-CoV-2 test results during its upcoming peak season.[9] We recommend further investigation to determine the prevalence of SARS-CoV-2 associated co-infection with broad diagnostic testing across the spectrum of patient illness severity and impact on patient outcomes within large sample sizes to draw statistical significance. Furthermore, we suggest further investigation of the immunosuppressive pathogenesis of the SARS-CoV-2 and probable beneficial immunity-enhancing and antimicrobial therapies. Appropriate de-escalation of antimicrobial medications should coincide with diagnostic testing and medication sensitivity results to maintain antimicrobial stewardship principles.

As the pandemic progresses, clinicians must be aware of COVID-19 morbidity and mortality attributable to pathogen co-infection and secondary infection. Based on the current research, patients with clinical deterioration and severe illness should alert suspicion of immunosuppression and possible co-infection. This knowledge should influence the prescription of immunotherapies and empiric, broad-spectrum antibiotics and antivirals. Broad microbial diagnostic testing may reduce morbidity and mortality within lymphopenic patients with higher risk for co-infections in the hospital setting, especially those with critical illness and extended hospital stays. Additional research is necessary to determine adequate COVID-19 co-infection targeted diagnostic testing panels, antimicrobial therapeutic guidelines, and beneficial immunological interventions. With this knowledge, hospital care guidelines for COVID-19 patients may include clinical solutions that could help reduce disease burden and increase patient survival.

Conflict of interest and financial disclosures

No authors have financial or non-financial actual or potential conflicts of interest.

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