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MINIREVIEWS

Imaging related to underlying immunological and pathological processes in COVID-19

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Abstract

The introduction of coronavirus disease-2019 (COVID-19) as a global pandemic has contributed to overall morbidity and mortality. With a focus on understanding the immunology and pathophysiology of the disease, these features can be linked with the respective findings of imaging studies. Thus, the constellation between clinical presentation, histological, laboratory, immunological, and imaging results is crucial for the proper management of patients. The purpose of this article is to examine the role of imaging during the particular stages of severe acute respiratory syndrome coronavirus 2 infection – asymptomatic stage, typical and atypical COVID-19 pneumonia, acute respiratory distress syndrome, multiorgan failure, and thrombosis. The use of imaging methods to assess the severity and duration of changes is crucial in patients with COVID-19. Radiography and computed tomography are among the methods that allow accurate characterization of changes.

Key Words: Coronavirus disease-2019; Ultrasound; Computed tomography; Magnetic resonance imaging; Ground-glass opacity; Acute respiratory distress syndrome; Cytokine storm; COVID-19 reporting and data system; High-resolution computed tomography; Severe acute respiratory syndrome coronavirus 2

Core Tip: The novel Coronavirus disease-2019 (COVID-19) infection may present as a multiorgan disease, with the lung being the most commonly affected target organ. The clinical presentation, course, and outcome of COVID-19 are heterogeneous. Various imaging modalities can be employed to evaluate different disease stages depending on the affected organ or system, with X-ray, computed tomography, and ultrasound being the most commonly used. Imaging plays an essential role in the primary diagnosis of all manifestations of the disease and its related complications, evaluating disease severity and follow-up. Proper utilization of different imaging modalities and interpretation of the key imaging findings are essential for effective patient management and treatment.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the etiologic agent of coronavirus disease-2019 (COVID-19), caused a global pandemic dominated by acute respiratory failure mortality. COVID-19 manifestations are heterogeneous and overlapping. Therefore many challenges in the diagnostics and management of COVID-19 are still present[1]. In addition, typical symptoms without positive proof of infection (PCR/antigen tests) make the diagnosis inconclusive. However, a variety of imaging methods and techniques can be used for this purpose. Furthermore, imaging is also essential for classifying and managing patients during the infection.

Typically, imaging studies are carried out to determine the disease stage and estimate the organ involvement and severity. However, the features, such as ground-glass opacities (GGO), consolidations, interstitial fluid shifts, *etc.*, seen on imaging are not unique to COVID-19. Therefore, a combination of the clinical picture and laboratory assessment is necessary to fully evaluate the patient's state[2].

In the era of COVID-19, radiology plays a crucial role in the disease work-up and follow-up. Initially considered a purely pulmonary process, COVID-19 turned out to be a multisystemic disease that requires a comprehensive imaging approach, involving all techniques and studying all anatomical areas. As the primary manifestation of COVID-19 is a pneumonia-like respiratory process, the radiology modalities most involved in its diagnosis and follow-up are chest imaging, mainly chest X-ray (CXR) and chest computed tomography (CT). Diagnostic imaging capabilities show that CT is more sensitive than the gold standard RT-PCR for diagnosing COVID-19[2,3]. At the same time, it is cheaper and can be performed faster. The CT examination can give a quick, accurate diagnosis of patients, as the PCR test requires hours, even days, to complete as the number of COVID-19 infection cases grow. However, this comes with a large radiation dose, where the capacity is still lacking in many countries[4]. CXR is ubiquitous worldwide, with a 30–70-fold lower amount of radiation. Despite its low sensitivity and specificity, it is commonly performed as an initial investigation in COVID-19[4,5]. It is the modality of choice in the intensive care unit to detect disease progression and assess the position of the individual resuscitation means.

The imaging findings both on CXR and CT can be invaluable, especially for an atypical or organizing pneumonia with bilateral, multifocal randomly scattered GGO, in subpleural, mainly peripheral distribution with thickened pulmonary interstitium giving a reticular pattern, broncho-vascular prominence, and consolidation with increasing severity in the more seriously ill patients[6]. High-resolution CT (HRCT) with its modern available software techniques is the method of choice for an initial examination, staging, and follow-up of patients with suspected COVID infection. CT has higher sensitivity and specificity than radiography. On CT, the main changes are GGO, which tend to be more in the periphery, crazy paving changes are seen in later stages of the disease, thickening of the interstitium is also seen as well as dilation of the terminal lung vessels[7]. For staging of the changes seen on CT, we use the COVID-19 reporting and data system (CO-RADS) classification. It is a standardized classification proposed by the Dutch Radiological Society. It has 6 levels of suspicion from CO-RADS 1 to CO-RADS 6. In CO-RADS 1 patients, the exam is normal or has non-infectious changes.

CO-RADS 2 patients have low levels of suspicion, and the visualized changes are consistent with infections other than COVID. CO-RADS 3 patients are those in which the changes are unclear, and there is an indeterminate level of suspicion. CO-RADS 4 and 5 staged patients have high and very high levels of suspicion for COVID- 19 infection, respectively. CO-RADS 6 patients are those who have typical changes and are PCR positive[8].

Chest ultrasound (US) plays a role in emergency settings as a COVID-19 screening technique. However, it is often of limited use as a highly operator-dependent method for lung disease. In addition, point of care echocardiography might have utility in hemodynamically unstable patients.

As stated above, various extrapulmonary manifestations have been reported, including in the gastrointestinal tract, brain, heart, kidneys, or muscles. The identification of most of these pathologies needs imaging, including abdominal CT, US, and magnetic resonance imaging (MRI). Imaging helps detect, diagnose, and assess the virus-induced injury and associated complications of the organs and systems affected by COVID-19. In suspected pulmonary thromboembolism (PTE) cases, CT pulmonary angiography (CTPA) may help correct the diagnosis. PTE is frequently observed in patients with more severe COVID-19 pneumonia involving mainly the segmental (90.2%) and subsegmental arteries (61.0%) of pulmonary segments affected by a consolidation pattern (67.6%)[9].

Furthermore, CXR, chest CT, and echocardiography can readily evaluate the signs of cardiac failure. However, myocardial injury can be best assessed using cardiac MRI. Potential neurovascular complications such as stroke, hemorrhage, or venous sinus thrombosis can be identified by non-enhanced head CT, and in the setting of a suspected infarct, a non-enhanced MRI of the brain can be performed for definitive assessment[10].

The disease may present as a multisystem hyperinflammatory syndrome in pediatric patients, currently termed pediatric multisystem inflammatory syndrome (PMIS). In children with PMIS, a broad spectrum of abdominal abnormalities can be detected by

both abdominal US and CT, with periportal and pericholecystic edema, gallbladder wall, and bowel wall thickening and dilatation, splenic infarcts, hepatosplenomegaly, right lower quadrant mesenteric lymphadenopathy, and free fluid in the pelvis being among the most commonly encountered abnormalities[10,11].

The COVID-19 pandemic has facilitated research on the implementation of artificial intelligence (AI), machine learning, and its subfield deep learning into imaging, which can be used as an essential adjunct or alternative to the diagnosis and follow-up assessment of progression and therapeutic development of the disease[12]. Deep learning is the most successful machine learning technique, which provides helpful analysis to study a large number of chest images that can critically impact the screening of COVID-19. AI algorithms have been developed to help with the early detection of COVID-19 both on CXR and CT. A deep learning model was also trained to discriminate between COVID and non-COVID pneumonia[13]. In line with this, CT pneumonia analysis (developed by Siemens Healthineers and partners) is another algorithm designed to automatically identify and quantify abnormal patterns in the lungs, enabling simple-to-use analysis of non-contrast chest CT scans for research purposes. The results could be used to analyze the severity and progression of abnormalities in patients exhibiting COVID-19 symptoms.

ASYMPTOMATIC DISEASE

A person infected with SARS-CoV-2 who has not developed any signs or symptoms of COVID-19 is defined as an asymptomatic case. Immunological features, including any of the innate immunity pathways (*i.e.*, natural killer (NK) cells, interferon, and other cytokine production), play a role at the onset of infection[14]. Stage I, or the asymptomatic incubation with or without detectable virus, is the period when treatment for improving immunity is given, such as the use of antiserums (ready-made antibodies from survivors), and is undoubtedly crucial[14]. However, due to the initial pathological changes in the target organs, sometimes imaging studies are the only way of detecting problems.

In asymptomatic individuals, this stage begins with inhalation of the SARS-CoV-2 virus replicating in the epithelial cells of the nasal cavity. It is well-known that the virus primarily uses the receptors for ACE2[15]; thus the first affected cells are ciliated cells. Therefore, PCR for SARS-CoV-2 RNA in nasal swabs can diagnose the virus at this point. The virus is then distributed in the lungs, digestive tract, reproductive system, *etc.*, while innate immune tolerance is minimal[15].

It has been shown that asymptomatic persons can be infectious and secrete SARS-CoV-2, promoting the dissemination of COVID-19 – a significant concern from an epidemiological point of view. Moreover, while asymptomatic, some patients present with substantial lung changes, for example, when they seek medical attention. This observation requires a stringent search and examination of the interactions of proven infected persons with COVID-19 to diagnose asymptomatic infections[14].

According to the literature and personal experience of our team, the incidence of asymptomatic occurring COVID-19 infection is much higher, about four times more common than symptomatic moderate to severe ongoing cases[16]. The described histological changes in the respiratory system in an asymptomatic case of COVID-19 infection based on autopsy and biopsy are exceptional[17]. The only

way to examine histological changes, mainly of the lung, in asymptomatic infection in the early stages of the disease is to take a biopsy for other pathological processes, "accidental" sampling of COVID-19, most often in the case of neoplastic diseases in which surgeries were performed for lung tumors at a time when superimposed infections were not recognized[16-20].

The main morphological changes found in routine histopathological examinations in the lungs are edema, proteinaceous exudate, focal reactive hyperplasia of pneumocytes, some with viral inclusions, and polymorphonuclear inflammatory infiltration composed mainly of lymphocytes and multinucleated giant cells. Hyaline membranes are scattered or not apparent in the early (asymptomatic) stage of COVID-19 infection, unlike in acute respiratory distress syndrome (ARDS). In addition, histologically, protein and fibrin exudates can be found in the lung parenchyma, as well as diffuse thickening of the alveolar walls, consisting of proliferating interstitial fibroblasts and type II pneumocystic hyperplasia, with random cell atypia and multinucleated giant cells, indicating varying degrees of the proliferative phase of diffuse alveolar damage. In some areas, an abundance of alveolar macrophages can be found together with type II pneumocystic hyperplasia[17,18,21].

Most patients with COVID-19 infection are diagnosed with pneumonia. The patients who have no symptoms could be transmitters. However, some asymptomatic patients progress quickly, even to ARDS. In asymptomatic patients, radiography has lower sensitivity and specificity than CT. In patients with slight changes, X-ray imaging can appear normal. This is why CT is the preferred method for diagnosis. Based on the published data, to date, almost all patients with COVID-19 have characteristic changes on CT[8,22].

The X-ray of a 60-year-old female patient is presented in Figure 1. The patient had no symptoms, but a positive PCR test was performed because her husband had COVID pneumonia. Her X-ray showed no abnormalities. This is a typical case of an asymptomatic patient with negative radiography.

We also present the CT scan of a 45-year-old male, shown in Figure 2. He had no symptoms but a positive PCR test because he had been in contact with a verified COVID-19 patient. Both lungs showed no abnormalities, no infiltrates, no GGO, and no pleural effusions.

Atypical clinical findings in pregnant women with COVID-19 may increase the difficulty in establishing the diagnosis. Recently, data have demonstrated that pregnant women are more vulnerable to severe COVID-19, the development of complications, and death. CT is the favored approach for detecting pulmonary involvement early, especially in asymptomatic cases[22]. In asymptomatic children or those with atypical presentation of COVID-19, CT may be beneficial, especially in sparing time while waiting for PCR results or false-negative results.

In the case of pregnant women or children, it is always preferred to perform CT instead of waiting because any delay in confirming the diagnosis and management of the disease can be fatal and can contribute to the spread of the virus. Furthermore, the CT results in asymptomatic COVID-19 are usually distinct from the normal appearance, where the most common findings are GGO and consolidations in the lungs[22].

MILD TO MODERATE CASES – TYPICAL COVID-19 PNEUMONIA

The onset of pneumonia in COVID-19 includes extreme antigen presentation, followed by enhanced production of C-reactive protein, D-dimer, and liver amino-transferases, accompanied by infiltrates in the lungs, representing involvement of antigen-presenting cells, T-helper cells, B cells, and NK cells[14].

The virus spreads across the airways in the respiratory tract and further to the gastrointestinal tract. It activates robust innate immune reactions. Nasal secretion, sputum or swabs contain SARS-CoV-2 but also early immune response molecules. Cytokine production during this stage may predict the clinical presentation and the subsequent disease course[23]. Significant amounts of interferon type I can confine the infection within the lungs, which is valid for approximately 80% of affected patients. Most of those with typical COVID-19 pneumonia on conservative symptomatic treatment can stay at home. However, about 20% of infected patients will progress to the next severe stage of the disease. Around 2% will develop life-threatening illnesses[23].

However, we have to keep in mind that COVID-19 pneumonia is a heterogeneous disease. It may include tracheobronchitis, vascular injury, and capillary microthrombi, along with inflammation[23]. Active injury leads to chronic and permanent lung trauma. The late effects of SARS-CoV-2 on the lungs have not been elucidated, but fibrosis development is suggestive.

Histologically, a hallmark of typical viral pneumonia is the interstitial nature of the inflammatory reaction. In addition, the most common morphologic changes in the lungs correlate with ARDS, which is described in the next section (Severe cases of COVID-19). On the other hand, no apparent morphological changes were observed in cardiac tissue due to the direct action of the coronavirus[17]. However, other findings established on necropsy material taken at autopsy are hypertrophy of cardiomyocytes with microscopic evidence of acute ischemia due to hypertensive heart disease and coronary artery atherosclerosis, but no other substantial damage[5].

Interestingly, we documented a case of sinus cavernosus thrombosis in a 49-year-old man hospitalized with COVID-19 pneumonia. One week after hospitalization, he lost vision in his right eye, and

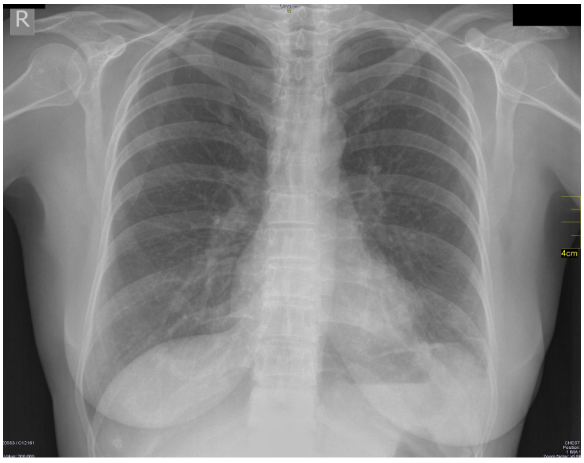

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Figure 1 Negative radiography of an asymptomatic patient.

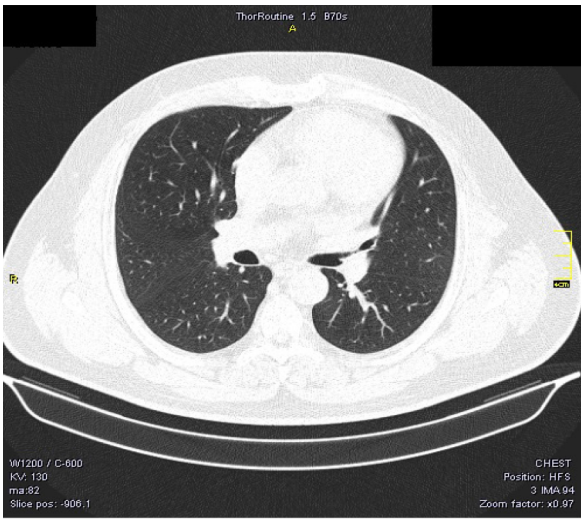

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Figure 2 Negative computed tomography of an asymptomatic patient.

presented with exophthalmos and swelling of the soft tissues around the eye. CT with contrast enhancement was performed immediately after the onset of symptoms. Thrombosis of the right sinus cavernosus is presented in Figure 3A and B.

COVID-19 pneumonia is classified as atypical pneumonia because it overlaps with the radiographic findings seen in interstitial pneumonia, including other coronavirus infections (SARS, MERS)[24,25]. Therefore, imaging methods are the first choice for diagnosing COVID-19 pneumonia including CXR and chest CT, with CT being a more sensitive and specific method than CXR, especially in the early stages of the disease[5].

Imaging findings on conventional radiography range from normal findings to diffuse changes in the lung parenchyma. Patients with multiple comorbidities are more likely to have bilateral and diffuse lesions. Findings considered as highly specific for COVID-19 pneumonia on CRX are GGO (Figure 4A) and areas of non-segmental consolidation of parenchyma (Figure 4B) with peripheral and caudal distribution[26]. These findings are most pronounced 10-12 d after the onset of symptoms[27]. Additional findings are confluent ill-defined patchy opacities (Figure 4C), an interstitial lung pattern, decreased lung attenuation, inhomogeneous and linear parenchymal opacities (Figure 4D), and often different features are combined.

On the one hand, for intensive care unit patients, CRX plays a crucial role in detecting disease progression and assessing the position of the individual resuscitation means - endotracheal tubes, drains, and venous sources as it is performed at the patient's bedside with a mobile X-ray machine[26].

On the other hand, CT is a vital component in the diagnosis of suspected COVID-19 infection. CT features vary with the patient's age, immunity status, disease stage, underlying diseases, and drug interventions at the time of scanning[28]. CT findings considered "typical" for COVID-19 pneumonia

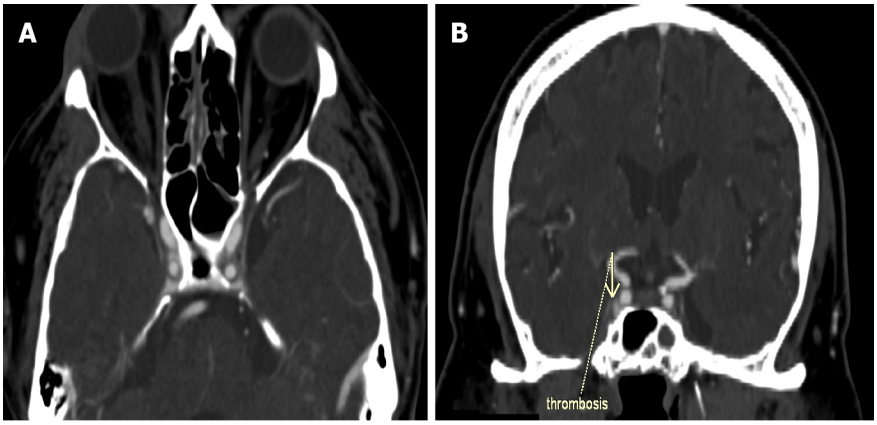

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Figure 3 Computed tomography with contrast enhancement. A: Axial slice of thrombosis of the right sinus cavernosus; B: Coronal reconstruction of thrombosis of the right sinus cavernosus.

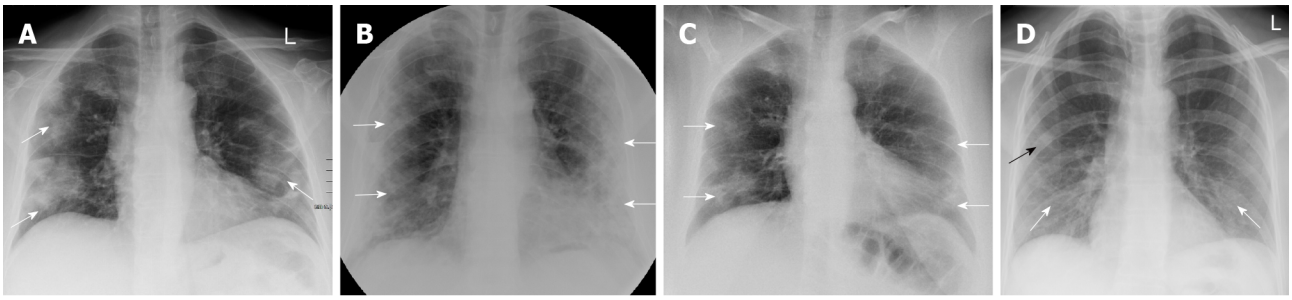

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Figure 4 Imaging findings on conventional radiography range from normal findings to diffuse changes in the lung parenchyma. A: Posteroanterior (PA) chest X-ray 7 d after symptom onset. Predominantly peripheral ground glass opacities in both mid and lower zones of the lungs (white arrows); B: PA chest X-ray 11 d after symptom onset. Bilateral dense peripheral and basal air space consolidation (white arrows) more pronounced on the left and loss of lung markings in the mid and lower zones on the left; C: PA chest X-ray 3 d after symptom onset. Bilaterally, approximately proportionally in the middle and lower lung lobes, confluent ill-defined patchy opacities are visualized (white arrows). There is a relative preservation of the central areas of the lungs; D: PA chest X-ray 4 d after symptom onset. Bilaterally, predominantly in the right lower lobe, fine linear opacities (white arrows) are seen. Additionally, small patchy ground glass opacities (black arrow) are visualized in the peripheral region of right middle lobe.

include GGO, parenchymal consolidations, and crazy-paving pattern (Figure 5)[29]. The changes are usually multifocal, bilateral with a peripheral subpleural distribution predominantly in the posterior segments of the inferior lobes[30,31].

A GGO is the most frequent and earliest finding defined as increased attenuation on CT, which does not obscure the bronchovascular structures. It can be observed in three typical patterns: rounded, linear and crazy paving[30] (Figure 6). In the area of GGO, widening of vessels and traction bronchiectasis are commonly seen[32,33]. GGO with reticular interstitial thickening, known as crazy-paving, is defined as thickening of the pulmonary interstitium with thickened interlobular septae and visualization of intralobular pulmonary septae[34]. Crazy paving may be seen with areas of GGO or consolidation in the subacute to chronic phase of the disease[35] (Figure 7).

By definition, consolidation is a homogenous increase in the lung parenchyma's attenuation with obscuration of the underlying vessels (Figure 8) and bronchi[34]. Therefore, it is considered a sign of disease progression, especially in the intermediate and late stages of the disease[30,34].

The distribution of lung abnormalities was recorded as predominantly subpleural (involving mainly the peripheral one-third of the lung), random (without predilection for subpleural or central regions), or diffuse (continuous involvement without respect to lung segments)[33] (Figure 5). Additional CT features of COVID-19 pneumonia include vascular dilation, bronchial wall thickening, and traction bronchiectasis within the areas of GGO, architectural distortion with reticular thickening and subpleural bands formation, halo sign, or reversed halo sign (Figure 9). Changes considered atypical for COVID-19 pneumonia include lobar or segmental consolidations, small nodules (centrilobular lung nodules and tree-in-bud opacities), pulmonary cavitation, lymphadenopathy, and the presence of pleural or pericardial effusions[34].

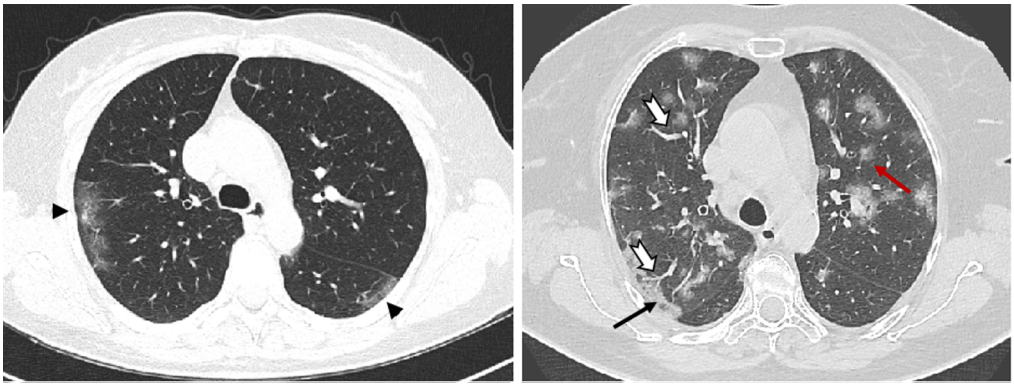

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Figure 5 Axial computed tomography images show different patterns of ground glass opacities – round (red arrow), linear (arrowheads) and crazy paving (black arrow) with vascular enlargement (thick white arrow) within ground glass opacities areas.

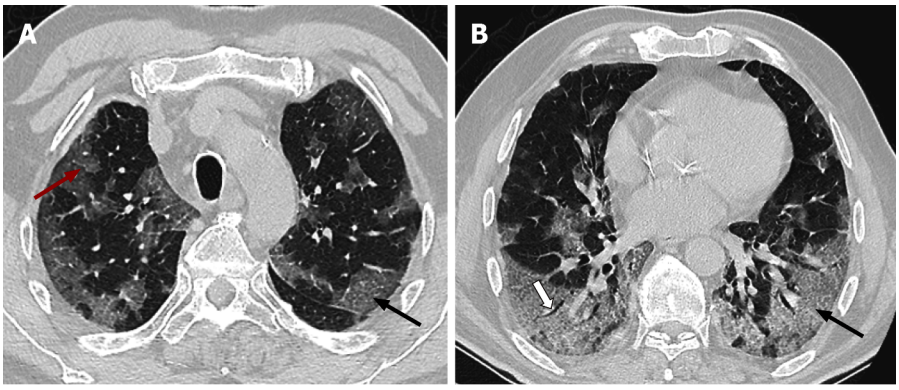

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Figure 6 Axial computed tomography scans (7 days after symptom onset) show peripheral bilateral ground-glass opacities. A: Superimposed reticular interstitial thickening (red arrow) within the ground-glass opacities (GGO) giving a 'crazy-paving' appearance (black arrow); B: Air bronchogram (thick arrow) within the GGO.

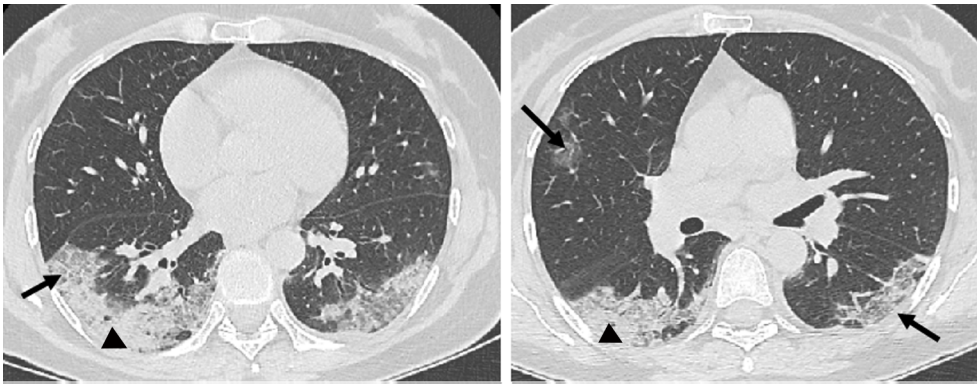

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Figure 7 Axial computed tomography images 11 days after symptom onset demonstrate bilateral confluent zones of ground-glass opacities and crazy paving (arrows) with subpleural, predominantly dorsal distribution and with superimposed areas of consolidation (arrowheads), more extensive in the right thoracic half.

CT findings also vary depending on the onset of clinical symptoms. Approximately four stages of COVID-19 on chest CT have been described: (1) Early stage (0-5 d after onset of symptoms) marked by either normal or predominantly GGO (Figure 10); (2) Progressive stage (5-8 d after onset of symptoms) marked by enhanced GGO and a crazy-paving look (Figure 11A and B); (3) Peak stage (9-13 d after onset of symptoms) characterized by progressive consolidation (Figure 11C and D); and (4) Late stage (≥

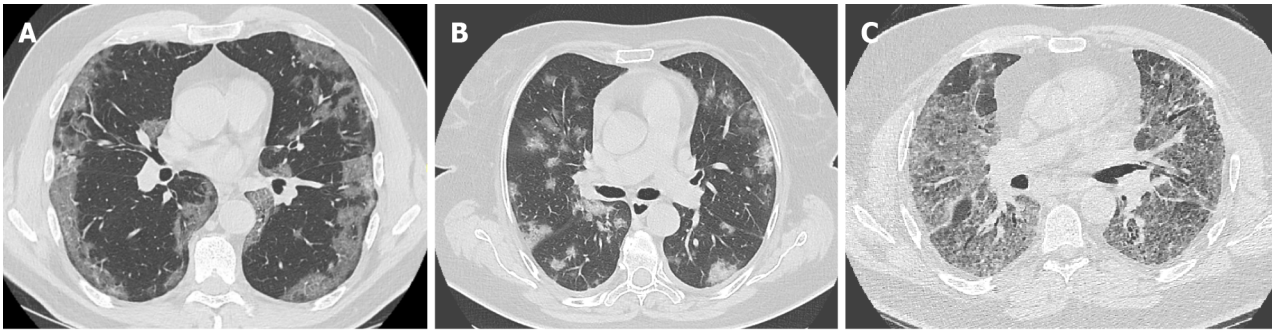

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Figure 8 Axial computed tomography images of patients with different time of symptoms onset showing various types of lung abnormalities distribution. A: Subpleural with involvement of peripheral one third of lungs; B: Random with central and subpleural location; C: Diffuse with confluence of changes and continuous involvement.

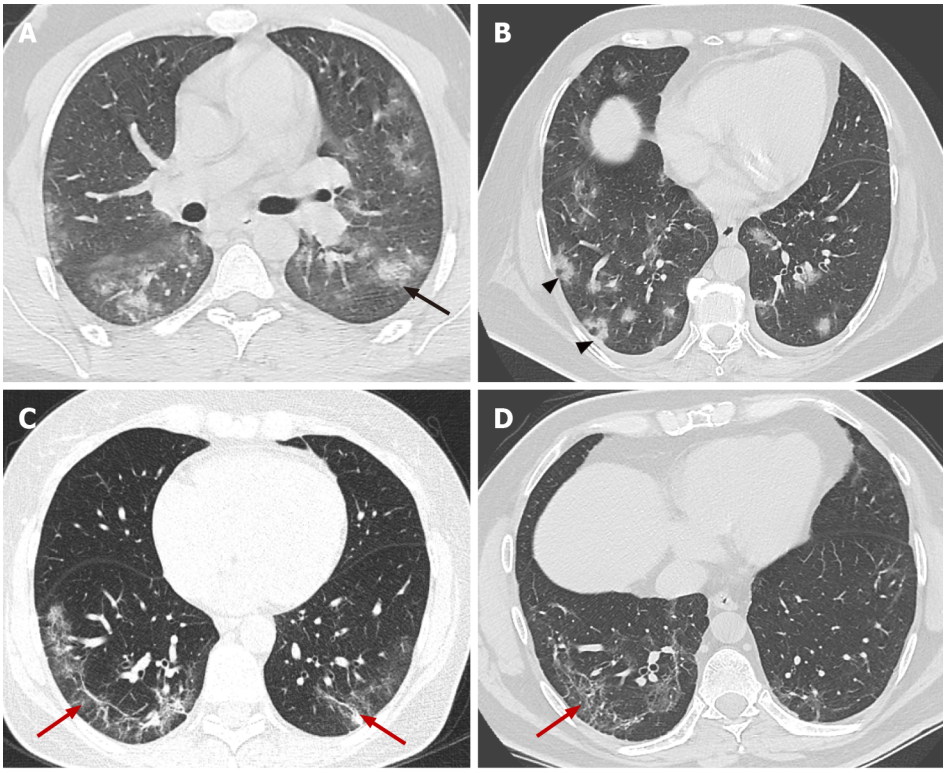

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Figure 9 Axial computed tomography images demonstrate features seen in organizing pneumonia. A: Ground-glass opacities (GGO) surrounding small area of consolidation, "halo sign" (black arrow); B: Central GGO surrounded by denser consolidation of crescentic shape, "reverse halo sign" (arrowheads); C and D: Architectural distortion with interstitial thickening and irregular fibrous bands (red arrows).

14 d after the onset of symptoms) denoted by a gradual decrease in consolidation and GGO, although signs of fibrosis (including parenchymal bands, architectural distortion, and traction bronchiectasis) may occur[31,36-39] (Figure 12). It should also be remembered that the temporal development and degree of lung defects are heterogeneous among different individuals, depending on the disease severity[31,36]. Similar findings can be observed in other viral types of pneumonia, pneumonia caused by Mycoplasma or Chlamydia, vasculitis, and connective tissue disease. Therefore, the clinical-laboratory correlation with the radiological finding is essential for the diagnosis of COVID-19 pneumonia.

CT scanning can be a useful tool in evaluating the individual disease burden[40]. The quantitative severity can be assessed using a visual method or software that determines the percentage of affected lung volumes using deep learning algorithms[41,42]. Furthermore, the severity of lung involvement on CT correlates with the severity of the disease. It can be measured by scoring the percentages of each of the five lobes that are involved and can range from 0 (no involvement) to 25 (maximum involvement) when all five lobes show more than 75% involvement[41] (Figure 13).

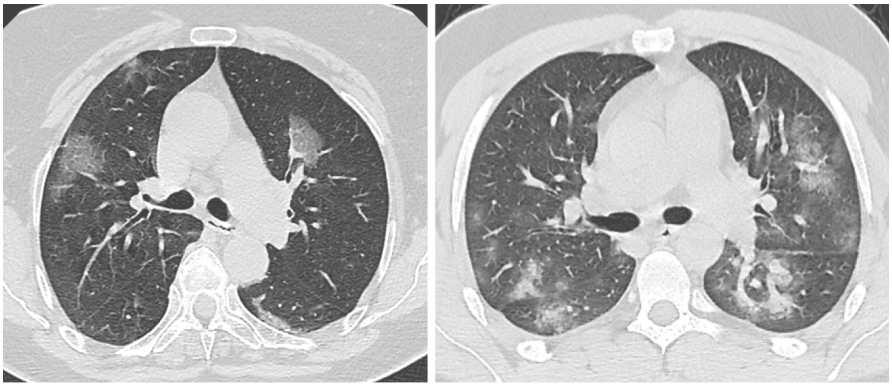

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Figure 10 Axial computed tomography images in two different patients 5 days after onset of symptoms show various degree of ground-glass opacities abnormalities (early stage of COVID-19).

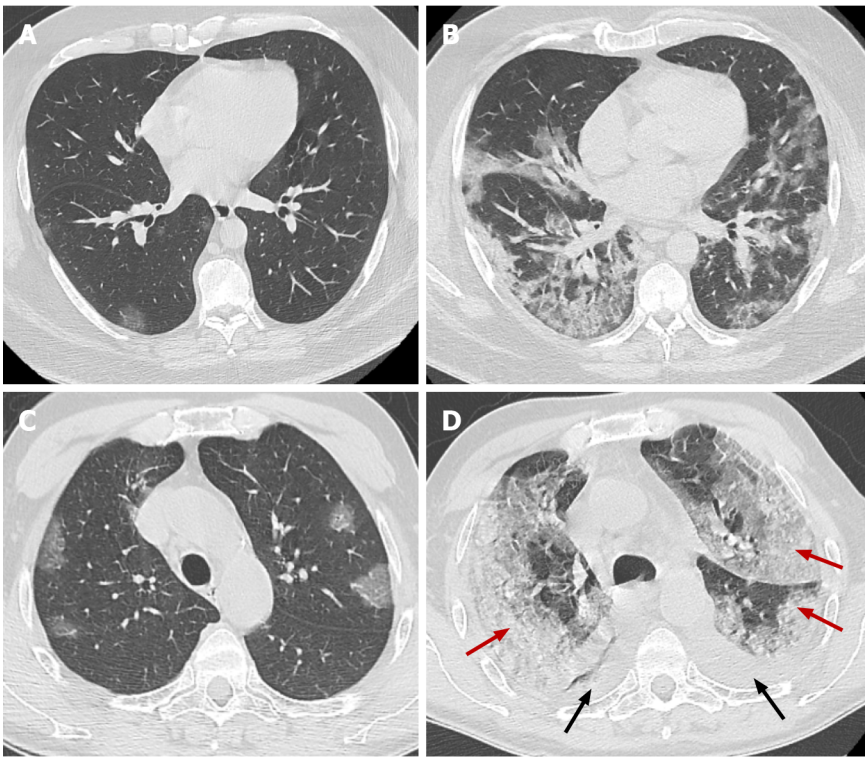

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Figure 11 Axial computed tomography images. Axial computed tomography images on day 2 (A) and day 10 (B) from onset of symptoms of patient with clinical worsening demonstrate increased ground-glass opacities with confluence, crazy-paving appearance and typical peripheral subpleural location, early (A) and progressive (B) stage. Axial images from CT on day 5 (C) and day 15 (D) from onset of symptoms of patient with pronounced clinical worsening demonstrate increased ground-glass opacities with confluence, crazy-paving appearance and extensive areas of consolidation (red arrows) with predominant posterior and lateral distribution. In addition, bilateral pleural effusions (black arrows) are noted, early (C) and peak (D) stage.

Lung ultrasound is increasingly used as a complementary method in diagnosing COVID-19 pneumonia. It allows assessment of both the lung parenchyma and the pleural space with the ability of bedside and real-time assessment of pulmonary function and changes. In addition, US can easily distinguish normally aerated from pathologically altered lung parenchyma. The most common findings observed in interstitial pneumonia are irregularly thickened pleura, B-lines, and subpleural consolidations of the parenchyma[43,44].

B-lines are vertical hyperechoic artifacts arising from the pleura that resemble a comet tail (Figure 14A) and move with the lung sliding. The increase in subpleural lung density (in the absence of consolidated tissue) may lead to the coalescence of many vertical artifacts in more extended echogenic patterns and a single homogeneous subpleural echogenic area can be seen. This phenomenon is known as "white lung"[44] (Figure 14B). In addition to B-lines, small, oval subpleural consolidations are visible in the parenchyma (Figure 14C). With disease progression to ARDS, the number of B-lines and

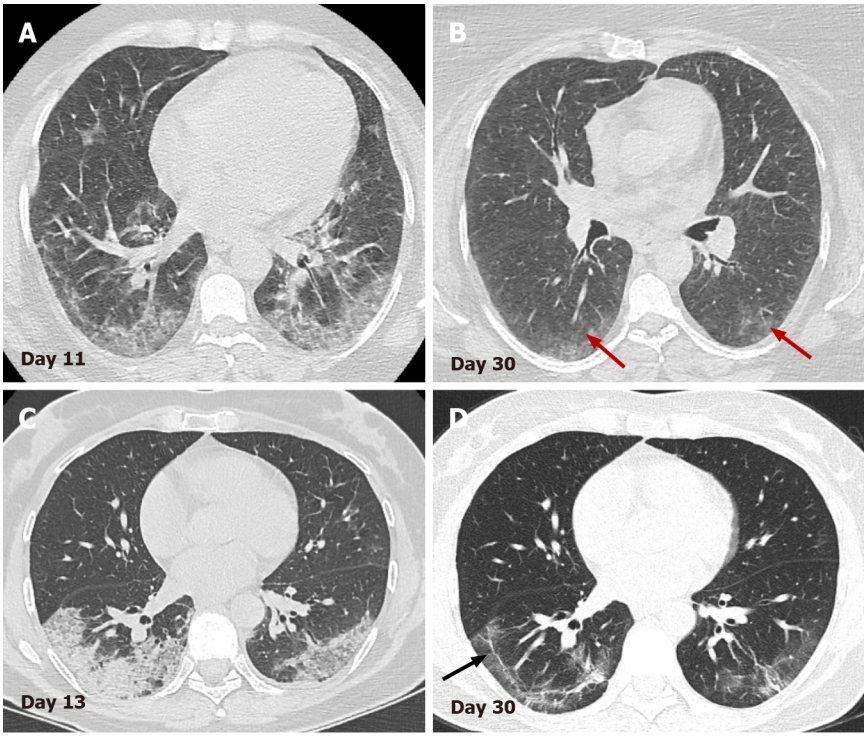

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Figure 12 Serial axial computed tomography images. Serial axial computed tomography images in two different patients show gradual decrease of GGO and consolidations in the late stage of disease (B) and (D). In patient 1 (A) and (B) advanced resorption of the abnormalities is seen with very low density GGO at the sight of previous lung changes (red arrows). In patient 2 (C) and (D) architectural distortion with fibrous bands parallel to the pleura (black arrow) and traction bronchiectasis are visualized (arrowheads).

parenchymal consolidations increases with the formation of sizeable gravitational consolidation[44], and the characteristic lung sliding is no longer visible. In the recovery phase of the disease, the consolidations and B-lines gradually disappear. Instead, A-line artifacts found in normally aerated parenchyma reappear and lung sliding is improved.

SEVERE CASES OF COVID-19

ARDS/cytokine storm

In some cases, COVID-19 pneumonia proceeds to the typical complications such as ARDS and cytokine storm. ARDS remains the most frequent immunopathological complication in SARS-CoV-2, SARS-CoV, and MERS-CoV viruses. The cytokine storm is one of the critical pathways for ARDS[45]. Both ARDS and cytokine storm represent an unregulated autoimmune inflammatory response arising from the release of significant quantities of pro-inflammatory cytokines (such as IFNg, IFNa, IL-1β, IL-6, IL-12, IL-18, IL-33, TNF-5-007, TGFβ, *etc.*) and chemokines (C-C Motif Chemokine Ligand 2 (CCL2), CCL3, CCL5, C-X-C motif ligand 8 (CXCL8), CXCL9, CXCL10, *etc.*) from immune or viral-infected cells[46]. Therefore, it is essential that these factors be detected and evaluated early. Thus, the appropriate therapy can be administered.

Histological examination of lung specimens in patients with severe infection is characterized by severe and extensive diffuse alveolar damage, fibromyxoid exudates, interstitial and intra-alveolar edema. However, in one of the cases described in the literature, the respiratory bronchial mucosa was intact, without evidence of squamous metaplasia. This is a significant difference compared with the pathology observed in the first epidemic of SARS[48]. In such patients, it is often found denuded with necrosis of type I pneumocytes and the formation of hyaline membrane and the proliferation of type II pneumocytes, which indicate ARDS in the acute stage without evidence of interstitial organization[17,21]. In addition, there is diffuse thickening of alveolar walls due to congestion, and patchy to mild mononuclear inflammatory infiltrate comprised of lymphocytes, macrophages, and some plasma cells. Perivascular lymphocyte aggregates have also been identified[47]. No eosinophils or neutrophils were identified, but if these are found, they usually suggest secondary bacterial infection[21,47]. Occasionally, in proliferated alveolar epithelial cells, viral inclusions in intranuclear and/or intracytoplasmic and multinucleated syncytial cells with atypical enlarged pneumocytes can be seen characterized by large eosinophilic nuclei with prominent nucleoli and granular cytoplasm (probably representing a viral

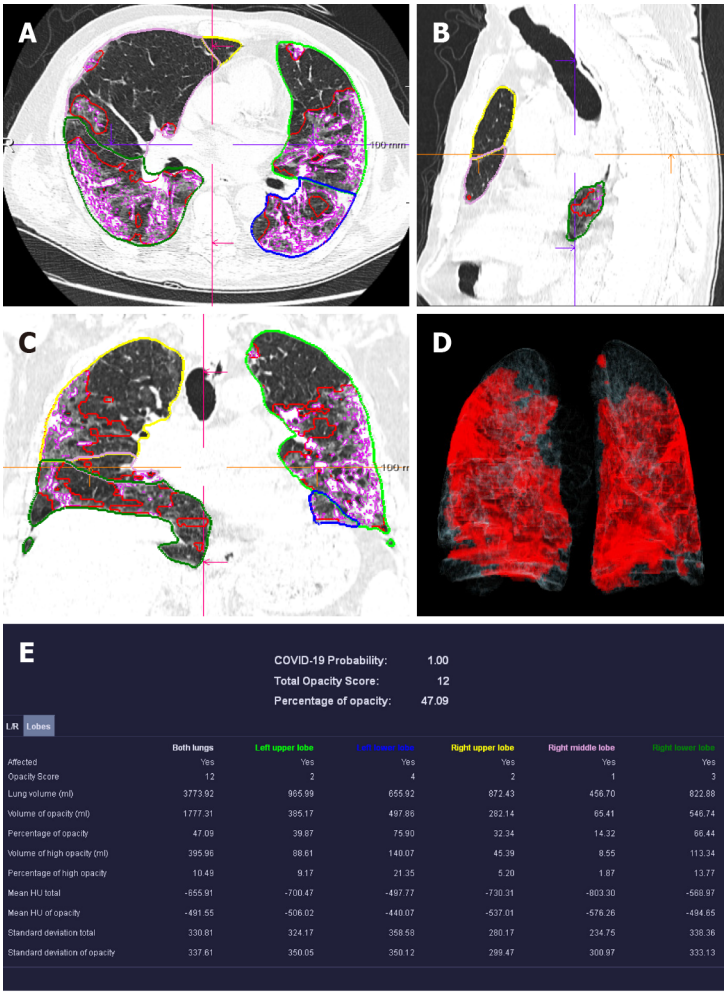

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Figure 13 Computed tomography pneumonia analysis (automated lung opacity analysis). A-C: Multi-planar reconstruction views overlaid with delineations of the opacities and the lungs; D: Volume rendering image showing a quick overview of the spatial distribution of the opacities; E: Table with measurements demonstrating relative ("percentage of opacities") and absolute volume of opacities, mean and standard deviation of HU values between lung parenchyma and the detected opacities, separately segmented quantitative results per left and right lung and per lung lobe.

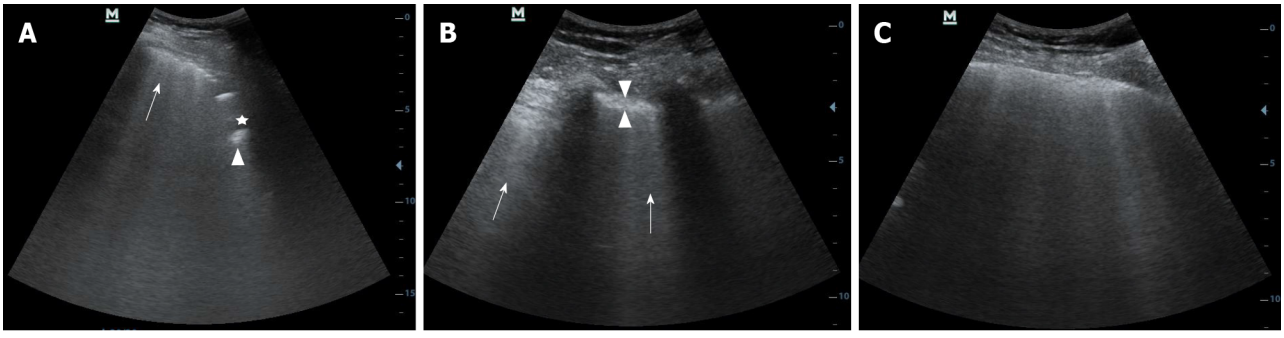

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Figure 14 Lung ultrasound image. A: Lung ultrasound image obtained with convex probe shows multiple B-lines (arrow) and subpleural consolidations seen as hypoechoic regions (asterix) that appear tissue-like with an irregular deep border (shredded fractal line) abutting the more aerated lung, which has echogenic artefacts (arrowhead); B: Lung ultrasound image obtained with convex probe. Multiple linear vertical artefacts - B-lines (arrows), and thickened pleural line (between arrowheads) are visualized; C: Lung ultrasound image obtained with convex probe. Multiple confluent B-lines are seen (white lung).

effect similar to that reported in the first SARS epidemic)[17,47-49]. Common in ARDS, pulmonary thrombi and microangiopathy may be noted within a few small pulmonary artery branches and pleural adhesions[21,47]. This thrombotic process may involve activation of megakaryocytes, probably those that are naturally found in the lung, with platelet aggregation and platelet formation, in addition to

fibrin deposition. The formation of small vascular thrombi in the lungs is often accompanied by focal alveolar hemorrhage[47,49].

With regard to imaging, COVID-19 related ARDS is diagnosed when a patient with confirmed COVID-19 infection meets the Berlin 2012 ARDS diagnostic criteria: acute hypoxemic respiratory failure; presentation within one week of worsening respiratory symptoms; bilateral airspace disease on CRX, CT, or US that is not fully explained by effusions, lung collapse, or nodules; and cardiac failure is not the primary cause of acute hypoxemic respiratory failure[50,51]. The risk factors for patients with COVID-19 to develop ARDS in the course of the disease are older age, concomitant conditions – most often hypertension and diabetes, and specific clinical symptoms on initial presentation such as marked dyspnea and fever ≥ 39°C[52]. However, clinical manifestations may be relatively mild regarding the severity of imaging findings in COVID-19[53].

Imaging studies have an essential role in the initial evaluation of the pattern and extent of lung involvement and in the follow-up of hospitalized patients. Chest radiographic findings of ARDS are non-specific and resemble those of typical pulmonary edema or pulmonary hemorrhage with diffuse, patchy or homogeneous, bilateral, and coalescent opacities[54] (Figures 15 and 16). Radiography in the first 24 h of deterioration may be unchanged[55]. CT imaging features depend on the phase of the disease[56]. In the early exudative stage (first week), CT scans usually display a non-homogeneous distribution and ventrodorsal gradient of density. More dense consolidations are observed in dependent regions, extensive GGO, and comparatively regular or hyper-inflated parenchyma (in the case of mechanical ventilation) in non-dependent areas[54] (Figure 17).

In the late fibrotic phase (over two weeks), CT appearance can be variable. Complete resolution may occur in some cases, but the coarse reticular pattern and ground-glass opacification in the anterior (non-dependent) part of the lungs are considered more typical CT features. Pulmonary cysts of varying sizes and bullae have also been reported, which probably developed due to prolonged ventilation[56]. Subsequent imaging studies can show the development of pneumomediastinum, pneumothorax (often hypertensive in mechanically ventilated patients), and subcutaneous emphysema[54] (Figure 18).

Multiorgan failure

The cytokine storm causes ARDS and multiorgan failure, leading to death in severe cases of coronavirus infections[45]. Although COVID-19 is known to cause pulmonary disease, including pneumonia and ARDS, various extrapulmonary manifestations of COVID-19 have been reported, affecting the gastrointestinal tract, brain, heart, kidneys, or muscles. Therefore, imaging helps to estimate the presence of complications and the extend of COVID-19.

Histologically, there is no noticeable viral cytopathic effect on the heart in the case of moderate infection on light microscopy[21,47]. However, liver biopsy specimens in patients with COVID-19 showed moderate fatty degeneration of hepatocytes and mild lobular and portal activity with slight interstitial mononuclear inflammatory infiltrates, indicating that the injury that could have been caused by either COVID-19 infection or drug-induced liver injury.

The pathogenesis of kidney injury due to COVID-19 is not well-described. However, it seems to be multifactorial, involving mechanisms related to systemic hypoxia, coagulation disorders, inflammatory changes, or even cell destruction due to viruses. According to the literature, renal impairment was related to multiple organ failure[57]. Furthermore, post-mortem examination of the kidney from a patient who died of COVID-19 infection demonstrated that viral antigens accumulated in the renal tubules, inducing acute kidney injury[57].

Histological examination confirmed that a diffuse proximal tubular lesion, loss of brush border, non-isometric vacuolar degeneration, and a small area of necrosis were observed. In addition, interstitial inflammation and hemorrhage were found in some of the published cases, probably due to secondary bacterial infection[58]. However, most of these findings are caused by comorbidities. The renal changes described above may be directly due to COVID-19 infection. These pathological observations may only provide a basis for further study and investigation of COVID-19[58].

The main focus of COVID-19 is respiratory system complications, which are also a leading factor determining the severity of the disease course. More and more is known about the pathophysiology of the disease and the mechanism of lung damage. However, the extrapulmonary manifestations of the disease remain unclear, which in some cases is decisive for the disease course[59]. Cardiovascular complications are receiving increasing attention, and according to various studies range from 30 to 78% [60-62]. Cardiovascular disease (CVD) is known to be one of the main risk factors for severe disease. A study of 44672 patients with COVID-19 infection in China showed that a history of concomitant CVD was associated with nearly five-fold higher mortality (10.5% *vs* 2.3%) than patients without a history of CVD[63]. In addition, increasing evidence suggests that the virus may directly affect the cardiovascular system and lead to complications such as myocarditis, acute coronary syndrome, arrhythmias, and venous thromboembolism[64].

Cardiomagnetic resonance imaging (CMR) represents the gold standard in assessing the structure and function of the heart and provides information on the tissue characteristics of the myocardium. That, together with its non-invasive nature and lack of ionizing radiation, makes it essential for both early diagnosis and monitoring of cardiac complications due to COVID-19.

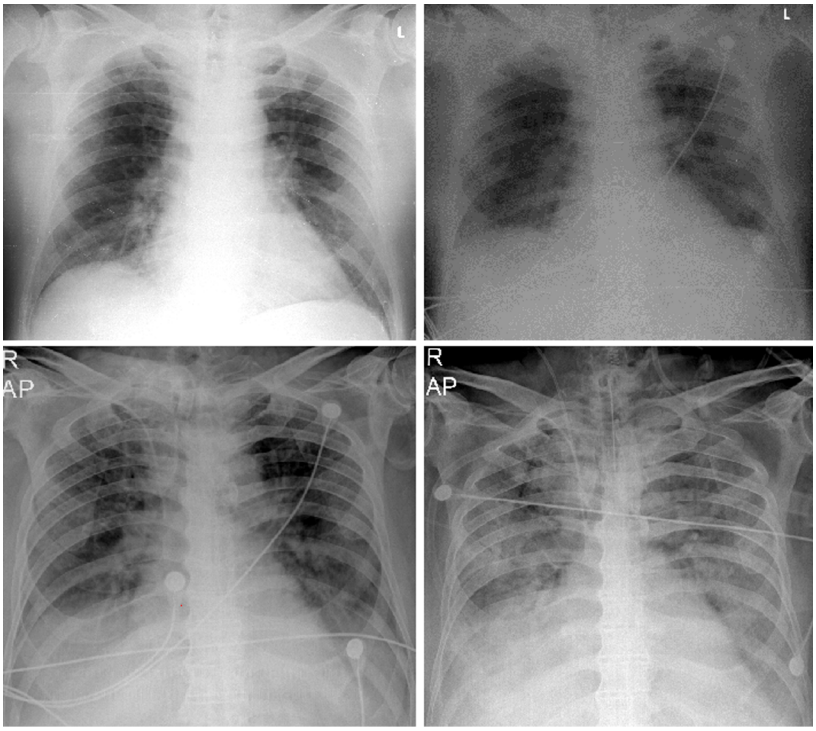

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Figure 15 Serial chest X-ray imaging of coronavirus disease-2019 infected 65-year-old man with rapid respiratory deterioration after symptom onset showing progression from lower lung predominant interstitial and airspace opacities on day 1 and day 3 to diffuse and worsening involvement with extensive airspace disease on days 4 and 6.

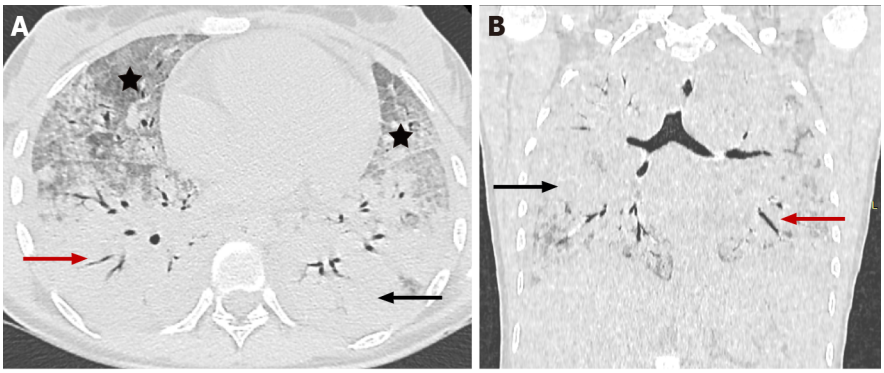

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Figure 16 Coronavirus disease-2019 related acute respiratory distress syndrome, early stage. Axial (A) and coronal (B) computed tomography images demonstrate widespread ground glass opacities (asterix) and large areas of consolidation (black arrow) with air bronchogram (red arrow) showing anteroposterior density gradient in both lungs.

The pathogenesis of cardiac damage is currently unclear. However, presumed mechanisms include direct viral invasion, cytokine-mediated injury, a mismatch between needs and oxygen supply, and ischemic impairment resulting from microvascular thrombosis[65,66].

The CMR findings in the acute phase of the disease reported so far are based on small groups of patients. The presence of myocardial edema is a characteristic feature observed in almost all reported cases[67-70]. In a review of 31 publications with 51 patients, only one patient did not have edema at baseline, and two patients developed reversible edema within two weeks[68].

The extent of edema varies - from diffuse to limited to separate segments of the myocardium - most often in the LV inferior wall regions, the mid inferoseptal regions, and the apical region[68]. Another characteristic CMR sign is late gadolinium enhancement (LGE) of the myocardium due to necrosis/fibrosis following nonischemic myocardial injury localized at the sub-epicardial and/or intramural regions in the non-coronary territory. The presence and extent of zones of LGE correlate with the prognosis[68]. An additional feature in the acute phase of the disease is diffuse hypokinesis of the left and/or right ventricle and, much less frequently, segmental hypokinesis[68].

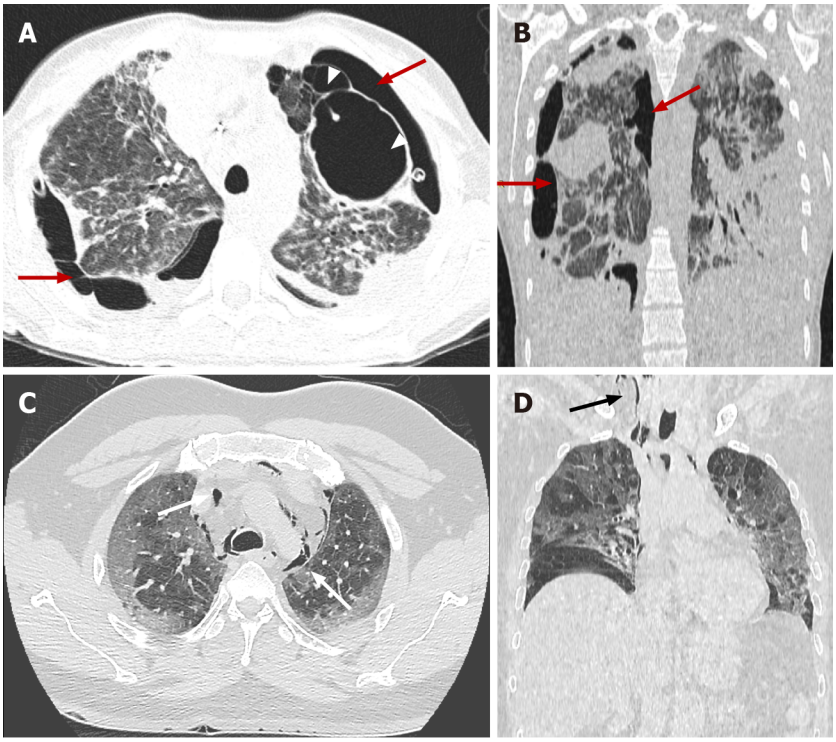

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Figure 17 Coronavirus disease-2019 related acute respiratory distress syndrome, late stage. Axial (A) and coronal computed tomography (CT) (B) images demonstrate typical complications in mechanically ventilated patients with subpleural bullae formation (arrowheads) and bilateral pneumothorax (red arrows). Axial (C) and coronal (D) CT images in different patient showing spontaneous pneumomediastinum (white arrows), extending into the neck as subcutaneous emphysema (black arrow).

Similar CMR findings - edema, necrosis/fibrosis, and impaired systolic function are also found in patients following COVID-19 infection[62,67,69-71]. Signs of persistent inflammation with prolonged T2 relaxation time due to edema, prolonged T1 relaxation time and LGE with a different distribution, and pericardial enhancement are seen. In some cases, reduced ejection fraction[62] is found.

Coagulopathy and thrombotic accidents

The cytokine storm is one of the leading causes of disseminated intravascular coagulation (DIC) affecting the entire organism. Pro-inflammatory cytokines, such as TNFa and IL-1, are able to inhibit endogenic anticoagulation. Inflammation injures the endothelium and results in activation of tissue plasminogen activator, which may explain the rise in D-dimer and fibrin degradation products[72,73]. In summary, COVID-19 is associated with a hypercoagulable disease and an elevated risk of thromboembolic complications.

The histological findings in organs other than lungs do not indicate significant changes directly related to COVID-19. However, the most commonly observed histological changes were due to thrombotic microangiopathy involving the lungs, spleen, and kidney. The most common complications which have been described in the literature include acute limb ischemia, aortic and mesenteric thrombosis, myocardial and brain infarction, and DIC[74].

A retrospective study on CTPA reported that pulmonary embolism in COVID-19 patients appears to be primarily distributed in the segmental arteries of the right lung[75]. Furthermore, only the determination of D-dimer and IL-6 at admission to CT scan appears to differentiate patients with pulmonary embolism from patients with a negative CT pulmonary angiogram. However, inter-individual heterogeneity calls for the establishment of cut-off values in COVID-19 patients in future research[76].

The pre-pandemic understanding of the predictive value of perfusion scintigraphy in assessing chronic thromboembolic disease and chronic thromboembolic pulmonary hypertension is well-established. Dhawan *et al*[75] proposed perfusion imaging as a triage tool for post-COVID-19 recovery. They suggested the potential of perfusion imaging to examine the *in situ* thrombotic small vessel signature of COVID-19. It is widely accepted that *in situ* thrombotic microbial pulmonary hypertension is present. Thus, lung perfusion imaging provides a primary triage instrument within the broader panel of investigations to enhance understanding of the natural history of thromboembolic phenomena in COVID-19 and distinguish hemodynamic sequelae from deconditioning dysfunctional breathing-related functional limitations. The authors also suggest that such imaging should be incorporated in routine post-COVID-19 follow-up pathways[75].

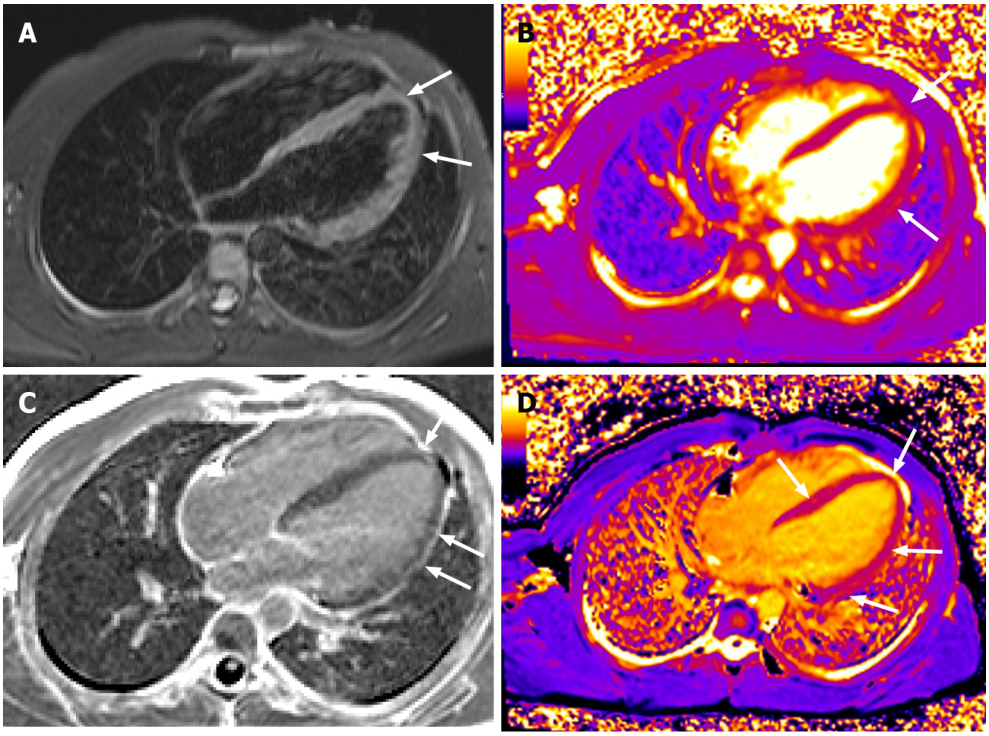

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Figure 18 Cardiac magnetic resonance imaging. Horizontal long axis TIRM image (A) displaying high signal in the myocardium at the apex and along the free wall of the left ventricle (LV). Horizontal long axis T2 (B) and T1 (D) mapping depicting prolonged T2 and T1 relaxation times at the same area and in the middle septum (D). Horizontal long axis image post gadolinium administration (C) showing late enhancement at the apex and along the free wall of LV. There is also pericardial enhancement along the free wall of LV.

CONCLUSION

Early observed changes in critical organs such as lungs, kidneys, blood vessels, *etc.*, might benefit the timely and appropriate treatment of COVID-19 to alleviate the complications and avoid fatal outcomes. Along with typical clinical and imaging results, atypical results are also beneficial. Early detection of pathological changes at different stages of life-threatening COVID-19 can improve patient management, treatment, and outcome.

FOOTNOTES

Author contributions: Boyapati A, Chervenkov L, Gulinac M, and Genova K performed the literature review; Chervenkov L, Ilieva E, Gulinac M, and Borisov Y collected and prepared the images from their institutes' database; Genova K, Ilieva E, and Chervenkov L provided the text and explanations of the images; Ilieva E and Velikova T wrote the draft; all the authors wrote additional sections in the paper according to their specialty; Velikova T revised the final draft; all authors revised and approved the final version of the article.

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ORIGINAL ARTICLE

Basic Study

Mutations of the *brpR* and *brpS* genes affect biofilm formation in *Staphylococcus aureus*

Allison Zank, Lillian Schulte, Xavier Brandon, Lauren Carstensen, Amy Wescott, William R Schwan

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Abstract

BACKGROUND

In the United States, *Staphylococcus aureus* (*S. aureus*) kills tens of thousands of individuals each year and the formation of a biofilm contributes to lethality. Biofilm-associated infections are hard to treat once the biofilm has formed. A new stilbene drug, labeled SK-03-92, was shown to kill *S. aureus* and affected transcription of two genes tied to a putative two-component system (TCS) we have named *brpR* (biofilm regulating protein regulator) and *brpS* (biofilm regulating protein sensor).

AIM

To determine if BrpR and BrpS regulate biofilm formation, *brpR* and *brpS* mutants were assessed using biofilm assays compared to wild-type *S. aureus*.

METHODS

A combination of biofilm and quantitative real-time-polymerase chain reaction assays were used. In addition, bioinformatic software tools were also utilized.

RESULTS

Significantly more biofilm was created in the brpR and brpS mutants *vs* wild-type cells. Quantitative real-time polymerase chain reactions showed the *brpS* mutant had differences in transcription of biofilm associated genes that were eight-fold higher for *srtA*, two-fold lower for *lrgA*, and 1.6-fold higher for *cidA* compared to wild-type. Bioinformatic analysis demonstrated that the *S. aureus* brpR/brpS TCS had homology to streptococcal late-stage competence proteins involved in cell-death, increased biofilm production, and the development of persister cells.

CONCLUSION

Our study suggests that *brpR/brpS* is a TCS that may repress *S. aureus* biofilm production and be linked to late-stage competence in *S. aureus*.

Key Words: Biofilm; Two-component system; Stilbene; *Staphylococcus aureus*; Late-stage competence; SK-03-92

Core Tip: *Staphylococcus aureus* is a primary cause of skin/soft tissue infections. In this study, we have shown that two previously uncharacterized genes, *brpR* and *brpS*, encode proteins that we believe comprise a two-component system that regulates biofilm formation in *S. aureus*.

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a significant pathogen of humans, causing more than 700000 skin/soft tissue infections, nearly 120000 bloodstream infections, and close to 20000 deaths *per* year in the United States[1-3]. Because drug resistance within this species continues to increase, new drugs are needed to treat human infections. Our research group has developed a new stilbene drug labeled SK-03-92 with efficacy against all Gram-positive bacteria that were tested, including methicillin-resistant *S. aureus*[4]. An mRNA microarray was performed on SK-03-92 treated *vs* untreated *S. aureus* cells to try to elucidate the mechanism of action of the drug[5]. From this microarray, the genes for a putative two-component system (TCS) (annotated as *MW2284/MW2285*) were the most downregulated at the transcriptional level. Moreover, transcription of the *srtA* gene (encoding sortase A) was upregulated and the *lrgA* gene encoding an anti-holin was downregulated following SK-03-92 treatment. Additionally, SK-03-92 treatment led to a high degree of persister cells and greater biofilm formation. Because of the effect on biofilm formation, the *MW22284* gene was labeled *brpR* (biofilm regulating protein regulator) and the *MW2285* gene was labeled *brpS* (biofilm regulating protein sensor).

Transcriptional changes of the *srtA* and *lrgA* genes as well as high numbers of persister cells suggested that SK-03-92 treatment may induce late-stage competence in *S. aureus*. Although competence allows DNA uptake to occur in heavily stressed cells, transformation is only one effect of bacterial competence. During early competence, which occurs prior to transformation, a large proportion of the stressed bacterial population die *via* holin-induced autolysis[6]. It is this phenomenon that supplies environmental DNA (eDNA) to the remaining cells for DNA uptake. Additionally, the surplus eDNA provides scaffolding for the rapid formation of a biofilm[7]. The final stage of natural competence is metabolic dormancy[8]. Current estimates show that only the youngest 1% of the original population survive to become a dormant cell. Thus, when faced with resource competition, a thriving bacterial colony has the ability to rapidly transform itself into a small group of latent (*i.e.* persister) cells living within a biofilm. These surviving cells re-emerge once environmental resources again become plentiful. This is one strategy used by bacterial cells to survive antibiotic challenge and re-infect the host[9].

The initiation of competence has been shown to rely on a symphony of genetic switches that begin to harmonize when short-sequence amino acids, known as competence stimulating pheromones (CSPs) bind to certain specific membrane proteins. The initiation of the CSP alarmone response has been well characterized in streptococcal species[10]. These membrane proteins are autoinducers that comprise one half of a specific TCS[11]. In *Streptococcus pneumoniae* (*S. pneumoniae*) and *Streptococcus mutans* (*S. mutans*), this response is initiated following the interaction with self-produced autoinducing pheromones known as CSPs, which are short, 14 residue peptides. The CSP is then received by the membrane bound sensor kinase ComD (*S. pneumoniae*)[12] or BrsM (*S. mutans*)[13]. Next, ComD or BrsM phosphorylate the cytoplasmic response regulator ComE (*S. pneumoniae*)[12] or BrsR (*S. mutans*)[13], which ultimately controls programmed cell death and persistence. In *S. aureus*, neither the CSP nor the TCS by which CSPs are received have yet been identified.

In this study, we have shown that mutations of the *brpR* and *brpS* genes in *S. aureus* strain Newman showed greater biofilm formation and transcriptional changes of the *srtA* and *lrgA* genes than wild-type *S. aureus*. Furthermore, we have used bioinformatic tools to show that the *brpR/brpS* TCS has homology to the BrsR/BrsM[13] and ComE/ComD[12] late-stage competence TCSs in *S. mutans* and *S. pneumoniae*, respectively. These findings suggest that the *brpR/brpS* TCS may be specific for the reception and resultant signal cascade of a molecule that induces late-stage competence in *S. aureus*.

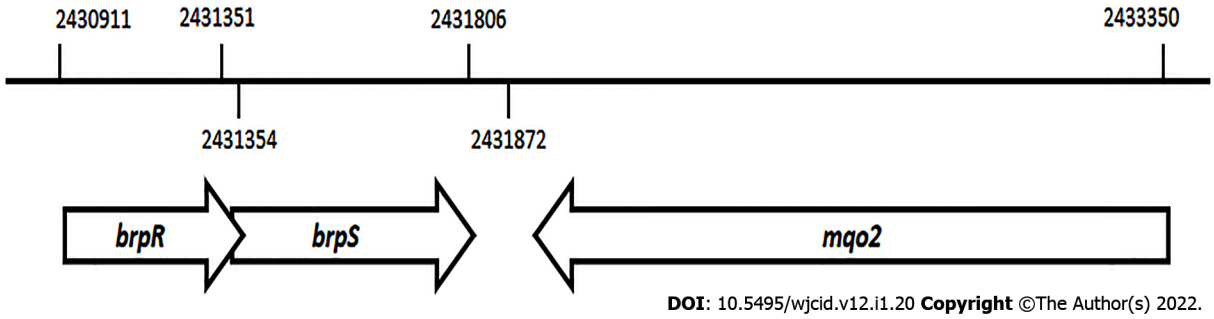

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Figure 1 Schematic representation of the chromosomal position and organization of the *brpR*, *brpS*, and *mqo2* genes in the *Staphylococcus aureus* strain MW2 genome.

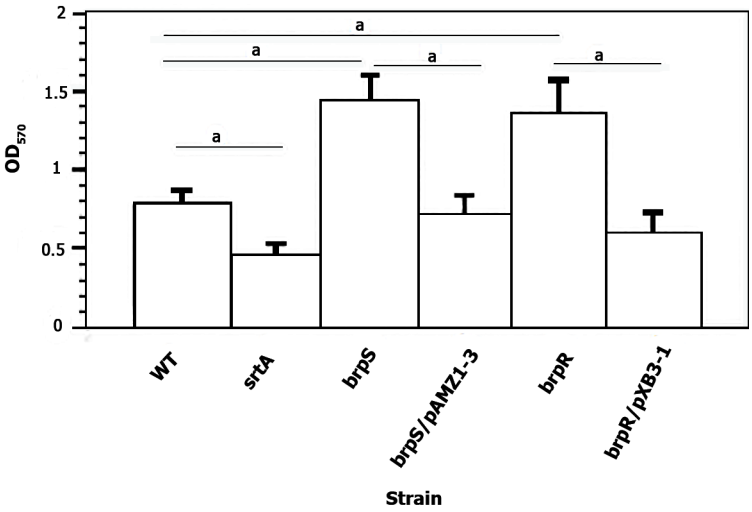

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Figure 2 Effect of *brpR* and *brpS* mutations and complementation on *Staphylococcus aureus* biofilm formation. All experiments represent the mean ± SD from five different runs done in triplicate for each strain. Biofilm formation was done on wild-type *Staphylococcus aureus* (*S. aureus*) strain Newman (WT, open column), *S. aureus* Newman *srtA* mutant (black column), *S. aureus* Newman *brpS* mutant (right striped column), *S. aureus* Newman *brpS* mutant/pAMZ1-3 (left striped column), *S. aureus* Newman *brpR* mutant (dark right striped column), and *S. aureus* Newman *brpR* mutant/pXB31 (dark left striped column). Differences were statistically compared by analysis of variance where $^{a}P < 0.001$.

MATERIALS AND METHODS

Bacterial strains, plasmids, and growth conditions

All of the bacterial strains and plasmids used in this study are shown in Table 1. The *S. aureus* parent strain Newman was isolated from a human infection[14]. The JE2 strain, created by the University of Nebraska Medical Center, is the *S. aureus* parent strain USA300 LAC CA-MRSA cured of its plasmids [15]. Strains NE272 (*brpS mutant*), NE671 (*brpR mutant*), and NE1787 (*srtA mutant*) are erythromycin-resistant (Em^R) mutants representing part of the Nebraska Transposon Mutant Library created by the University of Nebraska Medical Center by *mariner* transposon mutagenesis[15] and obtained from the Network on Antimicrobial Resistance in *S. aureus* (NARSA) strain repository (Table 1). The *E. coli* strain DH5α is a cloning strain with mutations that enable high-efficiency transformation[16]. *S. aureus* strain RN4220 is a transformation efficient strain of *S. aureus*[17].

To clone the *brpR* and *brpS* genes for complementation studies, plasmid pALC2073 was used. This plasmid carries ampicillin and chloramphenicol resistance genes, *E. coli* and Gram-positive origins of replication, and a *xyl/tetO* tetracycline inducible promoter[18].

All media was purchased from Thermo Fisher Scientific (Thermo Fisher Scientific, Pittsburgh, PA, United States). All antibiotics were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, United States). *E. coli* strains were grown in Luria (LB) broth shaken at 250 rpm at 37 °C or on Luria agar (LA) incubated at 37 °C. The *E. coli* strains carrying the pALC2073 plasmid were selected for in media containing 100 μg/mL ampicillin.

All *S. aureus* strains were grown in brain heart infusion (BHI) broth with 1% (wt/vol) glucose (BHI-G) or trypticase soy broth shaken at 250 rpm at 37 °C. Agar grown *S. aureus* cultures were passaged on BHI agar at 37 °C. The *mariner* transposon mutant strains were grown in media with 5 μg/mL of

Table 1 Bacterial strains and plasmids used in this study

Bacterial strain	Description	Ref.
E. coli		
DH5	Transformation efficient *E. coli* strain	[16]
S. aureus		
Newman	*S. aureus* clinical isolate	[14]
JE2	*S. aureus* USA300 MRSA strain	[15]
NE272	*S. aureus* JE2 *brpS*::*mariner* mutant	[15]
NE671	*S. aureus* JE2 *brpR*::*mariner* mutant	[15]
NE1787	*S. aureus* JE2 *srtA*::*mariner* mutant	[15]
RN4220	Transformation-efficient *S. aureus* strain	[17]
Newman *brpR*	*S. aureus* Newman *brpR*::*mariner* mutant	This study
Newman *brpS*	*S. aureus* Newman *brpS*::*mariner* mutant	This study
Newman *srtA*	*S. aureus* Newman *srtA*::*mariner* mutant	This study
Plasmids		
pXB3-1	pALC2073 plasmid with the *brpR* gene inserted	This study
pALC2073	Cloning vector with Ap^r, Cm^r, and Tc^r genes, and a Tc-inducible promoter	[18]
pAMZ1-3	pALC2073 plasmid with the *brpS* gene inserted	This study

erythromycin (Em^5). *S. aureus* strains carrying the pALC2073, pXB3-1, or pAMZ1-3 plasmid were selected for in media containing 10 μg/mL of chloramphenicol (Cm^{10}). To induce the *xyl*/*tetO* promoter on the pXB3-1 and pAMZ1-3 plasmids, tetracycline at 0.25 μg/mL was added to the growth medium.

Transduction of S. aureus

The *brpR*::*mariner*, *brpS*::*mariner*, and *srtA*::*mariner* mutations were transduced into strain Newman using the a ϕ80a bacteriophage[19]. The transductants were then selected for on BHI agar containing Em^5. All mutations were verified by polymerase chain reaction (PCR) and biofilm assays.

Construction of brpS and brpR complementing plasmids

The *brpR* complementing plasmid was constructed using the pALC2073 backbone[18]. Isolation of pALC2073 plasmid DNA followed the manufacturer's instructions for the Qiagen QiaPrep plasmid isolation kit (Qiagen, Germantown, MD, United States). The full-length coding region of the *S. aureus* strain MW2 *brpR* gene was PCR amplified using the MW2284I/MW2284M primers (Integrated DNA Technologies, Coralville, IA, United States; Table 2) and the following PCR conditions: 35 cycles, 94 °C 1 min, 57 °C 1 min, 72 °C 1 min. *S. aureus* strain MW2 chromosomal DNA served as a template. The *brpR* DNA was amplified to have a *Kpn*I site on the 5′ end and an *Eco*RI site on the 3′ end. PCR amplified *brpR* gene product was digested with *Kpn*I and *Eco*RI (New England Biolabs, Ipswich, MA, United States), and then ligated with *Kpn*I/*Eco*RI cut pALC2073 plasmid DNA using T_4 DNA ligase (New England Biolabs). Ligated DNA was transformed into *E. coli* strain DH5α cells[16]. Transformants were selected on LA containing 100 μg/mL ampicillin, and one resulting plasmid, plasmid pXB3-1, was used for further experiments.

A recombinant *brpS* complementing plasmid was also constructed. Isolation of pALC2073 plasmid DNA used the same plasmid isolation kit described above. The full-length coding region of the *S. aureus* strain MW2 *brpS* gene was PCR amplified using the GEX-5XB/GEX-5XC primers (Table 2) and the following PCR conditions: 35 cycles, 94 °C 1 min, 57 °C 1 min, 72 °C 1 min. *S. aureus* strain MW2 chromosomal DNA was used as the template. The *brpS* DNA was amplified to have a *Kpn*I site on the 5′ end and an *Eco*RI site on the 3′ end. PCR amplified *brpS* gene product was digested with *Kpn*I and *Eco*RI (New England Biolabs), and then ligated with *Kpn*I/*Eco*RI cut pALC2073 plasmid DNA using T_4 DNA ligase (New England Biolabs) immediately downstream from the tetracycline-inducible *xyl/tetO* promoter on pALC2073. Ligated DNA was transformed into *E. coli* strain DH5α cells[16]. Transformants were selected on LA containing 100 mg/mL ampicillin, and one resulting plasmid, plasmid pAMZ1-3, was used for further experiments.

Plasmid DNA from *E. coli* was purified with a Qiagen Plasmid Miniprep Kit (Qiagen) and electroporated into the *S. aureus* strain RN4220[20] using a GenePulser, (Bio-Rad, Hercules, CA, United States) under the following conditions: 100 W capacitance, 25 mF resistance, 2.5 kV charge voltage, 4 s.

Table 2 Primers used in this study

Primer	Sequence
GEX-5XC	5′- CCTAGGAGATCTCTTTCTGTC -3′
GEX-5XB	5′- GTTAATTTTACTAAACTTAAG -3′
MW2284I	5′- GAGCAGGTACCATGATGAAACTCAATTTATTTATCAATGCAAAAG-3′
MW2284M	5′- CAGCGAATTCTCAATGGTGATGGTGATGTATTGATAATCGCTCCTTTATAGATTTTAAAA -3′
CidA1	5′- TGCAACGATACATGTTCCTATG -3′
CidA2	5′- CTACAACTAGGAATCATCATTGTG -3′
LrgA1	5′- GCATCAAAACCAGCACACTTT -3′
LrgA2	5′- GACTTCGCCTAACTTAACAGC -3′
SaFtsZ1	5′- GGTGTAGGTGGTGGCGGTAA -3′
SaFtsZ2	5′- TCATTGGCGTAGATTTGTC -3′
SrtA1	5′- TCGCTGGTGTGGTACTTATC -3′
SrtA2	5′- CAGGTGTTGCTGGTCCTGGA -3′

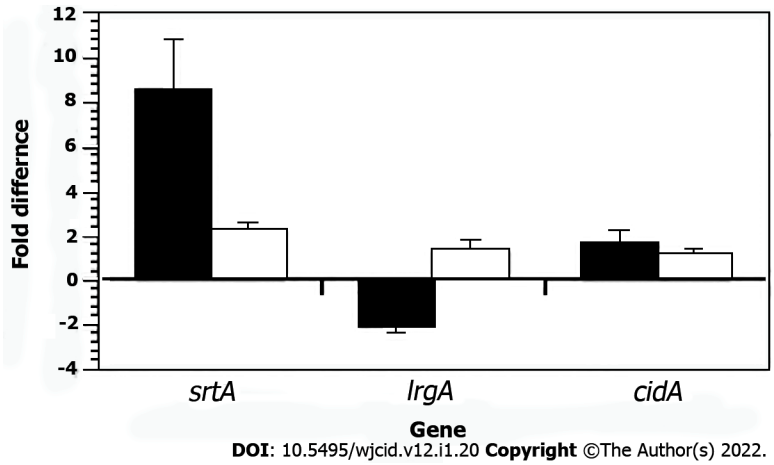

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Figure 3 Quantitative reverse transcribed-polymerase chain reaction results of *Staphylococcus aureus* Newman *cidA*, *lrgA*, and *srtA* transcription in wild-type bacteria (standardized to 0) compared to a *Staphylococcus aureus* Newman *brpS* mutant (black column) and the complemented *brpS* mutant (white column). The data represents the mean ± SD from three separate runs.

Transformants were selected for on BHI agar containing Cm^{10} after one hour of expression in BHI broth. Finally, plasmid DNA was re-isolated from one *S. aureus* strain RN4200 transformant carrying either the pXB3-1 or pAMZ1-3 plasmid using the method noted above with one alteration. The *S. aureus* cells were incubated with 50 µL of lysostaphin (10 mg/mL; Remel, San Diego, CA, United States) for 60 min at 37 °C prior to the first step to facilitate lysis of the staphylococcal cells. Each isolated plasmid DNA sample was then cut with the *Kpn*I and *EcoR*I restriction endonucleases to verify the insertion. The *S. aureus* strain Newman was then transformed with 10 mL of pXB3-1 or pAMZ1-3 plasmid DNA using electroporation as outlined above and transformants selected for on BHI agar containing Cm^{10}.

Biofilm assays

To determine the effect of the *brpR* and *brpS* mutations on the ability of *S. aureus* Newman to form a biofilm, biofilm assays were performed[21]. Briefly, cultures of the *S. aureus* were grown at 37 °C with shaking (250 rpm) overnight in BHIG broth with the appropriate antibiotic(s). Each strain was then diluted 1:100 in BHI-G and 220 µL of the solution was placed in microtiter wells in triplicate in a 96-well microtiter plate. The microtiter plates were statically incubated for 24 h at 37 °C to allow a biofilm to form. Each well was then rinsed three times with sterile water. The biofilms were then allowed to settle (10 min), stained with crystal violet dye (0.1% wt/vol) for 10 min, and then washed with sterile water. After allowing the well contents to dry fully in a sterile hood, the dried contents were incubated in 33% acetic acid at room temperature for 30 min. The contents of the well were vigorously curettaged. The optical densities were measured on a SpectraMax M3 96-well microtiter plate reader (Molecular

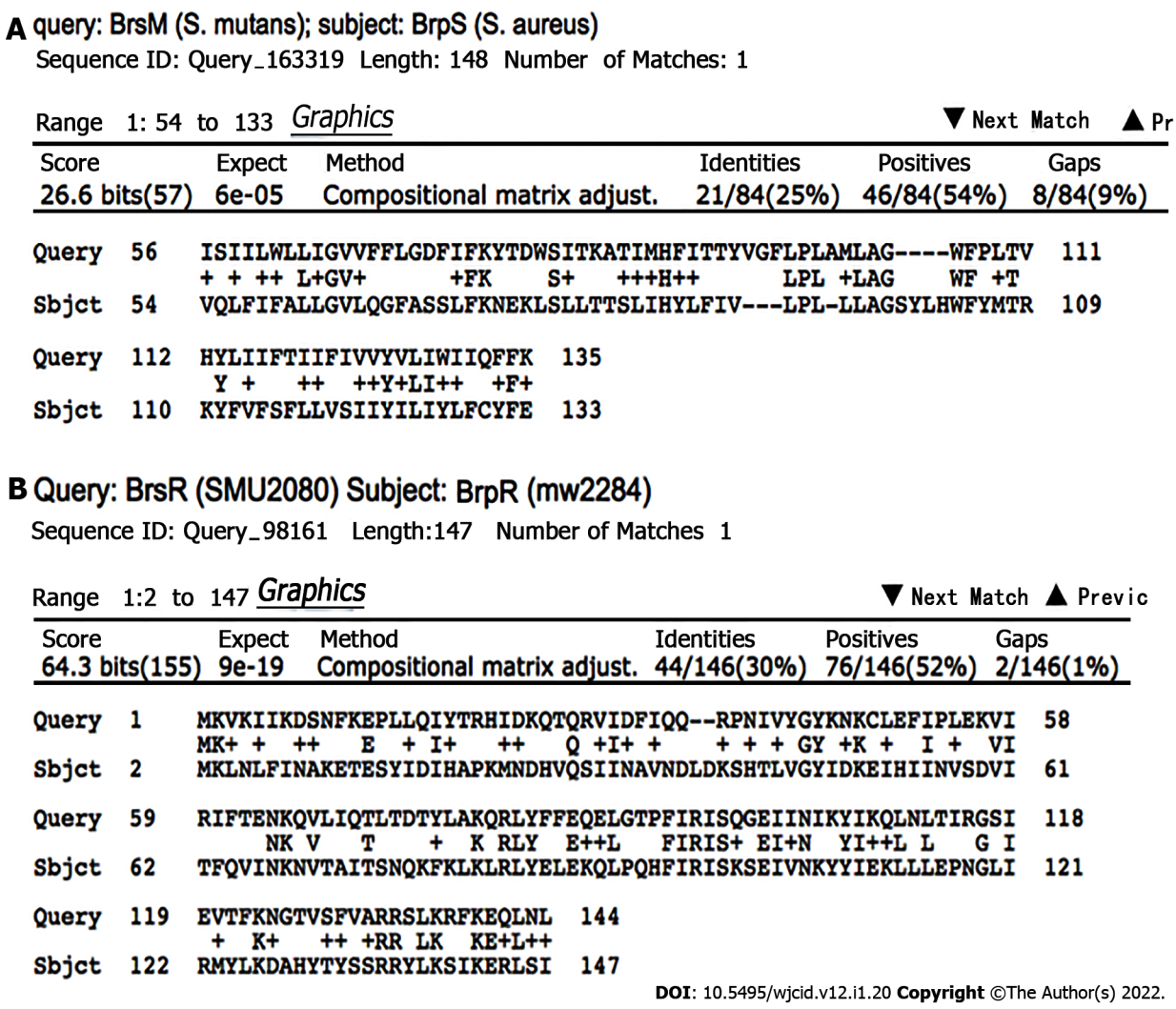

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Figure 4 Bioinformatic comparison of the BrsRM proteins of *Streptococcus mutans* with the BrpRS proteins of *Staphylococcus aureus*. A: BrsM, a two-component system membrane protein responsible for activating competence in response to sensing competitor organisms within a niche, shares sequence similarity with BrpS; B: BrsR, which is the cognate response regulator to BrsM, shares sequence similarity with BrpR. BLASTp NCBI. Algorithm parameters: Max target sequences = 100, automatically adjusted parameters for short input sequences, expect threshold = 10, word size = 3, max matches in a query range = 0, matrix = BLOSUM62, gap costs = 11 existence and 1 extension, and a conditional compositional score matrix adjustment.

Devices, San Jose, CA, United States) at an optical density of 570 nm. In addition to wild-type Newman cells, Newman *brpR* and Newman *brpS* mutant strains as well as *brpR* and *brpS* mutants containing the pXB3-1 or pAMZ1-3 plasmids were tested and compared with a Newman *srtA* transposon mutant strain that served as a negative control. To achieve statistical significance, the biofilm assays were performed a minimum of five times in triplicate for each strain.

Quantitative real-time PCR

Total RNA was isolated from *S. aureus* strains grown to early logarithmic phase in BHI broth with shaking (250 rpm) incubated at 37 °C using a High Pure RNA Isolation kit ΔΔ(Roche Diagnostics, Indianapolis, IN, United States) with an additional lysostaphin treatment step to help lyse the *S. aureus* cell walls and a DNase I digestion to digest contaminating DNA. To confirm RNA concentration and ensure the integrity of each RNA sample, an aliquot of each RNA sample was analyzed on a Nanodrop machine (Thermo Scientific, Waltham, MA, United States) and electrophoresed through 0.8% agarose gels. The cDNA for each strain was then synthesized from 2 μg of total RNA according to manufacturer's instruction using a First-Strand Synthesis kit (Life Technologies, Carlsbad, CA, United States). All quantitative real-time PCR (qRT-PCR) trials were performed according to manufacturer's instruction using the iTaq Universal SYBR Supermix kit (BioRad, Hercules, CA, United States). Oligonucleotide primers that targeted the *ftsZ*, *srtA*, *lrgA*, and *cidA* genes were synthesized (Table 2) by Integrated DNA Technologies. To perform qRT-PCR, the minimum information for publication of quantitative real-time PCR experiments guidelines were followed and the *ftsZ* housekeeping gene was used as a standardization control[22]. All replicates were performed at least three times on a CFX96 qPCR instrument (BioRad, Hercules, CA, United States) under the following conditions: 94 °C, 20 s; 55

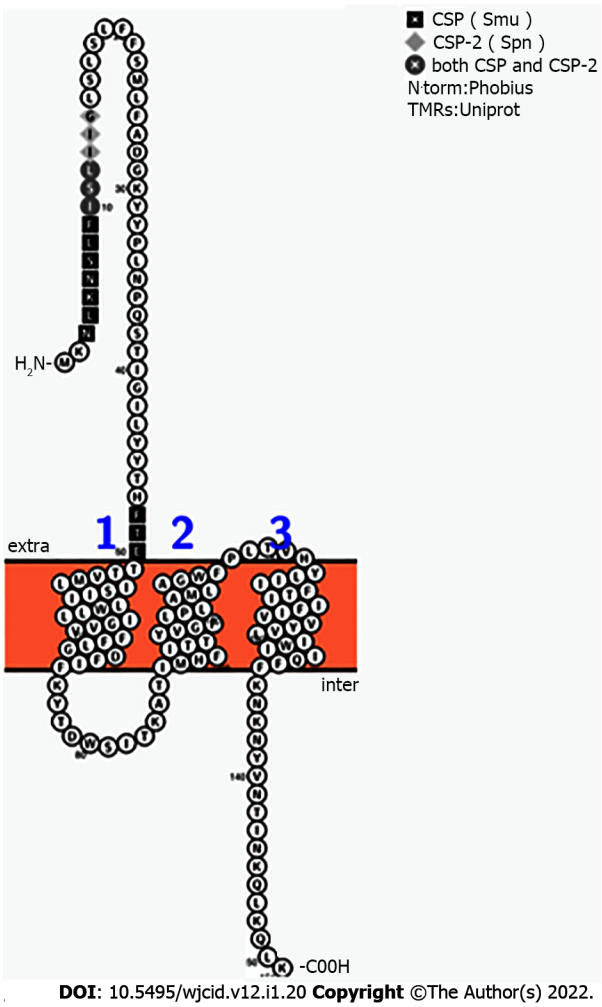

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Figure 5 Highlight of amino acid residues shared by competence stimulating pheromone, competence stimulating pheromone-2, and BrpS. The predicted exterior segment of BrpS, which spans N'-1-MKNLKNSLFISLIIGLSLSLFFSMLFADGKYYPLNPQSTIGILYYTHFT-50-C', was compared to competence stimulating pheromone (CSP) and CSP-2 by BLASTp. The competence stimulating peptides of *Streptococcus mutans* (1-SGSLSTFFRLFNRSFTQA-18, CSP) and *Streptococcus pneumoniae* (1-EMRISRIILDFLFLRKK-17, CSP-2) has 56% similarity to CSP and 30% similarity to CSP-2. CSP: Competence stimulating pheromone.

°C, 30 s; and 72 °C, 1 min for 35 cycles. The level of target gene transcript from each strain was estimated against the *ftsZ* gene standard curve. Additionally, crossover points for all genes were standardized to the crossover points for *ftsZ* in each sample using the $2^{-\Delta\Delta CT}$ formula[23].

Bioinformatic tools

The sequenced genomes of *S. aureus* strains MW2 and Newman used in this study are publicly available on GenBank (NCBI, genome assembly ASM1126v1)[24-26]. The protein annotations for all of the bacterial strains included in this study were found on BioCyc or GenBank[26,27]. BioCyc was also used to search for brpRS homologs downstream of the *mqo2* gene. The UniProt Consortium was used to obtain amino acid FASTA sequences[28]. Domain motifs were sought using NetPHOS, ExPASy, Prosite, and GenomeNet[28-30]. Protter was used to two-dimensionally visualize brpS and brsM[31]. I-TASSER and PyMOL were used together to three dimensionally visualize BrpR and BrpS[32,33]. I-TASSER and PyMol were also used to visually verify DNA binding in residues predicted by DP-Bind[34]. Finally, protein sequence homology analyses were performed by BLASTp (NCBI) with the following parameters: Max target sequences = 100, automatically adjusted parameters for short input sequences, expect threshold = 10, word size = 3, max matches in a query range = 0, matrix = BLOSUM62, gap costs = 11 existence and 1 extension, and a conditional compositional score matrix adjustment[35].

Statistical analysis

Calculation of the means, standard deviations, and paired Student's t-tests were performed using Microsoft Excel. $P < 0.05$ were considered significant.

RESULTS

Alignment of the brpS/brpR genes

An alignment of the *brpR* and *brpS* genes that encode the BrpR and BrpS proteins is seen on the *S. aureus* MW2 genome sequence (Figure 1)[24]. These genes overlap in a unidirectional in-tandem sequence. The overlapping *brpRS* genes lie just 66 base pairs upstream from the *mqo2* gene, encoding one of the two malate: Quinone oxidoreductases (MQO2) produced by *S. aureus*. The bi-functional MQO2 protein is able to generate oxaloacetate through an oxidation of malate as well as donate electrons to the electron transport chain[36].

Mutations in the brpR and brpS genes cause greater biofilm formation in S. aureus

To confirm biofilm formation was linked to BrpR and BrpS, individual *brpR* and *brpS* mutations were moved to the *S. aureus* strain JE2 background[15] to the *S. aureus* strain Newman *via* transduction. Biofilm production of both mutants was compared to the unmutated wild-type *S. aureus* Newman strain. Significantly more biofilm material was produced by the *brpS* and *brpR* mutants (1.8-fold and 1.73-fold higher, respectively, $P < 0.001$) compared to wild-type. Complementation of the *brpR* and *brpS* mutants caused biofilm expression to either return to wild-type levels or there was less biofilm material formed (Figure 2). The *srtA* mutant displayed a 1.73-fold decline in the biofilm forming ability compared to the wild-type strain ($P < 0.001$). This suggested that the putative BrpRS TCS may repress *S. aureus* biofilm production.

Transcription of srtA and lrgA are regulated by a brpS mutation

Previously, we showed *srtA* transcription was elevated and *lrgA* transcription was lower after SK-03-92 treatment of *S. aureus* cells compared to untreated cells[5]. The *srtA* gene encodes sortase A[37] and the *lrgA* gene encodes an anti-holin[38] that are important for the formation of biofilms[39,40]. Total RNA was collected during the mid-exponential growth phase from the *S. aureus* Newman *brpS* mutant, *S. aureus* Newman *brpS* mutant containing the pAMZ1-3 plasmid, and wild-type S. *aureus* Newman cells Each RNA sample was converted to cDNA for qRT-PCRs analysis. The *brpS* mutant displayed 8.5-fold higher *srtA* transcription ($P < 0.008$), 2-fold lower *lrgA* transcription ($P < 0.016$), and 1.6-fold higher *cidA* transcription ($P < 0.43$) *vs* the wild-type strain (Figure 3). Complementation of the *brpS* mutation caused *srtA* transcription to drop to a 2.2-fold increase, a 1.3-fold increase in *lrgA* transcription, and a 1.2-fold increase in *cidA* transcription. These results demonstrated that transcription of some biofilm-associated genes was regulated by a mutation in the *brpS* gene.

BrpR/BrpS homology to other TCS proteins

BrpR and BrpS homologs were identified by BLAST analyses in multiple Gram-positive bacterial pathogens, including *Bacillus cereus, Clostridioides difficile, Enterococcus faecalis, Lactobacillus species, Staphylococcus haemolyticus, Streptococcus pneumoniae* ComD/ComE, *and S. mutans* BrsR/BrsM as well as three other bacterial species (*Escherichia coli* YehT/YehU, *Mycobacterium tuberculosis* YehT/YehU, and *Chlamydia trachomatis*)[26,35]. Of these, the *S. mutans* BrsR/BrsM TCS that senses CSP and then induces late-stage competence showed the highest homology (Figure 4)[12].

Putative structures of the BrpS protein

S. aureus BrpS and *S. mutans* BrsM have a similar arrangement of reactive residues. Lysine, serine, threonine, histidine, tyrosine, and glutamic acid residues were illuminated on a 2-dimensional Protter-generated image of each protein (Figure 5)[29]. This mapping suggested that BrpS and BrsM are partitioned into distinct functional domains separated by the membrane. Functionality appears to occur at the intercellular loop (staphylococcal N'-76-KYTDWSITKAT-86-C'), at the extracellular loop (staphylococcal N'-108-PLTVHY-113-C'), and within a single reactive residue near the membrane at K135 (staphylococcal) within the C'-terminal tail region.

Additionally, the region at the N'-terminus of brpS displays sequence homology with the secreted *S. mutans* and *S. pneumoniae* CSPs (Figure 6). The segment of brpS spanning the regions from N'-1-MKNLKNSLFISLIIGLSLSLFFSMLFADGKYYPLNPQSTIGILYYTHFT-50-C' showed 56% similarity with CSP (*S. mutans*, 1-SGSLSTFFRLFNRSFTQ A-18) and 30% similarity to CSP-2 (*S. pneumoniae*, 1-EMRISRIILDFLFLRKK-17).

DISCUSSION

S. aureus causes 65% of biofilm-associated infections *per* year[40,41]. Biofilms provide a defense against host immune defenses as well as most antibiotics. An understanding of what regulates *S. aureus* biofilm formation could lead to treatment options that target this process in *S. aureus*.

Both sortase A (SrtA) and antiholin (LrgA) are important *S. aureus* proteins needed for creation and maintenance of biofilms. Sortase A promotes the covalent anchoring of surface proteins to the cell wall

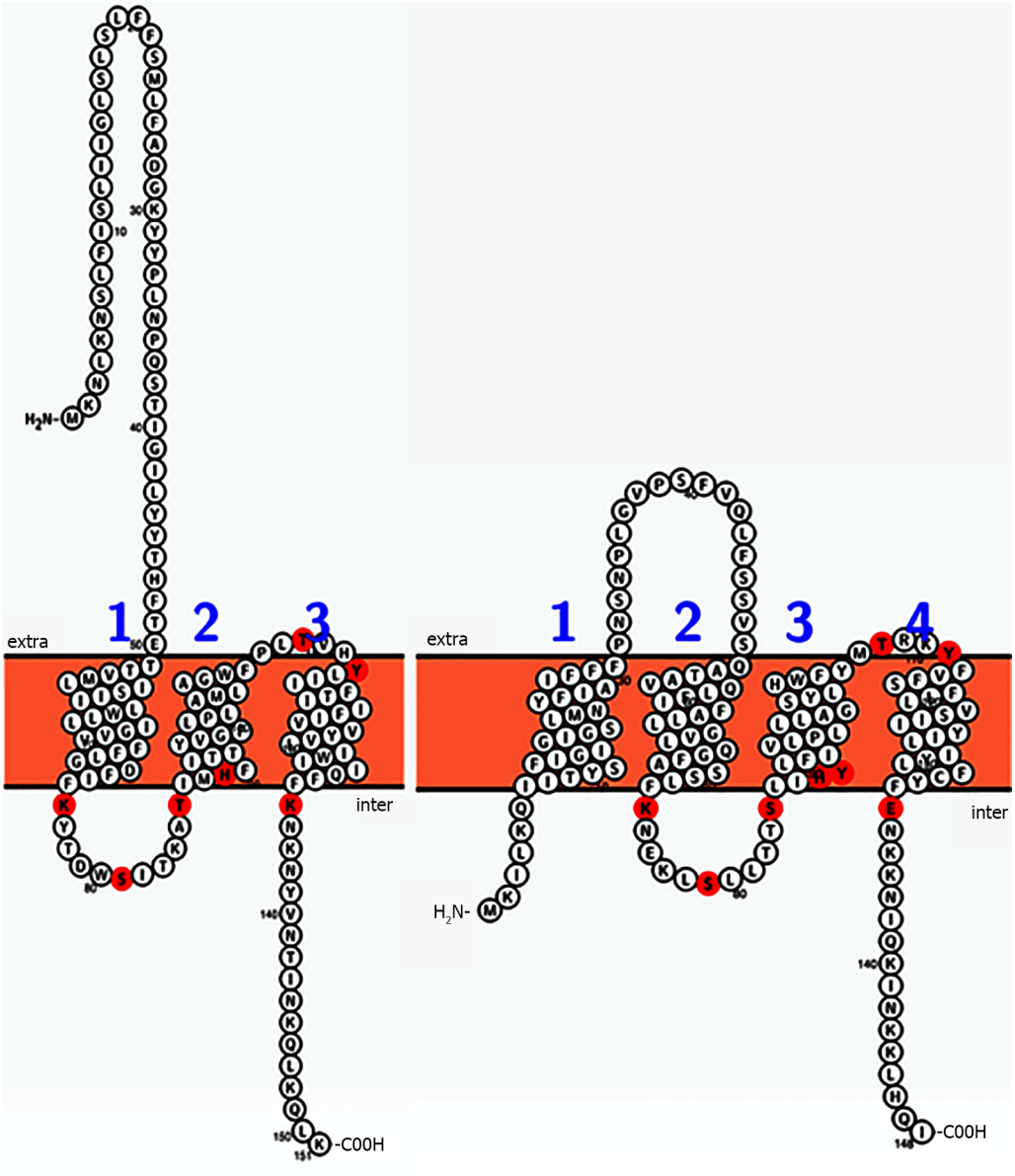

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Figure 6 Comparison of the sequences and predicted topologies of the putative two-component system membrane sensor BrpS (left, *Staphylococcus aureus*) and BrsM (right, *Streptococcus mutans*). According to this prediction, residues likely to be reactive (red) are topologically arranged in similar loci among both proteins. Intra is proposed to correspond with the cytoplasmic space, and extra is proposed to correspond with the extracellular milieu of the cell. Figures generated by Protter.

of *S. aureus*[42] that are important in the first stage of biofilm formation. Cell death releases eDNA that is tied to holin/antiholin action. An integral part of mature *S. aureus* biofilms is eDNA[7]. The function of the antiholin LrgA is to prevent cell autolysis by complexing with CidA holins[38,43].

In this study, we have shown that mutations in either the *brpR* or *brpS* gene cause an increase in biofilm formation as well as transcriptional changes of the *srtA* and *lrgA* genes, which are linked events. Previous studies with transposon mutants of what was an uncharacterized gene, that we have named *brpS*, displayed better biofilm formation than the wild-type strain[44,45]. Strains with a mutated *lrgA* gene have also been shown to produce increased levels of biofilms[46]. Another study has shown that cell lysis caused eDNA to be rapidly produced that could act as a scaffolding for newly forming biofilms [10].

The *in silico* data; biofilm results with the *brpR* and *brpS* mutants; and the data from the transcript abundance changes of the *lrgA*, *srtA*, *brpR*, and *brpS* genes suggest that BrpR/BrpS comprise a TCS that may be involved in late-stage competence. From the *in silico* analysis, we speculate that the BrpR protein (that possesses an apparent LytTR DNA binding-motif[47]) may repress *srtA* transcription. Other proteins that have LytTR motifs, such as BrsR and ComE, have been shown to have multifunctional activities tied to activation and repression[47-49]. Further analysis is required to show that BrpR is capable of binding to this region.

From the data presented, we speculate that BrpS is a receptor for a CSP-like pheromone secreted by *S. aureus* as a response to competition for resources. The leader peptide of BrpS may function to competitively antagonize the extracellular receptor portion of BrpS from the CSPs of competitive species, such as *S. mutans* and *S. pneumoniae,* that inhabit the human upper respiratory tract.

A number of previously completed studies focused on biofilm production and bacterial cell viability due to interactions with CSP-like pheromones. A study by Zhang *et al*[49] demonstrated that within *S. mutans* there was a 76.3% decline in cell viability and biofilm mass increased by 89.3% following the addition of CSP to bacterial growth media[49]. In addition, supernatant collected from *S. mutans* that was co-cultured with *Aggregatibacter actinomycetemcomitans* caused a 1.3-fold rise in biofilm production within *S. mutans*[50]. Ample evidence of cell death after CSP exposure has been documented by several studies, however, biofilm production has not been normalized to the viable bacterial cells that remain [51-53]. Nevertheless, a number of Gram-positive bacterial species show cell viability and subsequent biofilm production correlate with the level of CSP added to the media. Further studies should be done to assess the actual increase in biofilm formation by taking into account the findings that competence is accompanied by massive cellular death.

We also believe that there may be a connection between metabolic dormancy and the BrpR/BrpS TCS. If malate production is interrupted after BrpR binds to the sigma factor binding sites, malate conversion to oxalacetate would be halted. As a consequence of this interruption, any acetyl groups generated by acetyl-CoA would not interact with citrate within the citric-acid cycle. Thus, too much acetyl-CoA would arise within the cell. Because these functional groups would be liberated, it is possible that there would be an epigenetic modification and BrpR would be rapidly released from the *srtA* gene enhancer region. By freeing BrpR from the *srtA* gene enhancer region, additional BrpR molecules would be available to interact with sigma factors, blocking transcription of *brpRS* that would lead to even less transcription of the *mqo2* gene. The work by Zhang *et al*[54] used profiling of lysine acetylomes in *S. aureus* and *E. coli* to identify a sequence motif, which supports our idea that BrpR may epigenetically block DNA-binding[54]. As part of that study, 412 proteins and 1361 lysine sites were cross-referenced against each other, which led to a conserved motif, N'-RLYELExQLxxxFIRISKxxEIVN-C', being identified. BrpR has this conserved motif, which is very well conserved among a number of bacterial species. By shutting down malate expression, persister cells could form suddenly as a response to late-stage competence or treatment with the SK-03-92 drug. Thus, BrpR repression of malate production could be connected to formation of persister cells that is a feature of late-stage competence.

CONCLUSION

Our study suggests that BrpR/BrpS is a TCS that may repress *S. aureus* biofilm production and be linked to late-stage competence in *S. aureus*.

ARTICLE HIGHLIGHTS

Research background

Staphylococcus aureus (*S. aureus*) is a primary cause of skin/soft tissue infections. Biofilm formation is a key component of *S. aureus* pathogenesis. Thus, an understanding of what regulates biofilm formation in *S. aureus* is important.

Research motivation

We were interested in characterizing two open reading frames that we thought were tied to biofilm formation in *S. aureus*.

Research objectives

Determine if mutations in the *brpR* and *brpS* genes affected biofilm formation and what the respective proteins had homologies with.

Research methods

We used biofilm assays and quantitative real-time-polymerase chain reaction (qRT-PCR) analysis to test *brpR* and *brpS* mutants compared to the parent strain of *S. aureus*. Bioinformatic tools were used to determine what roles the BrpR and BrpS proteins may play in *S. aureus* cells.

Research results

The biofilm and qRT-PCR analyses demonstrated that mutations in the *brpR* and *brpS* genes affected biofilm formation in *S. aureus* and led to transcriptional differences in key biofilm-related genes as compared to the parent strain. Further, the BrpR and BrpS proteins share homologies with proteins

involved in late-stage competence in streptococcal species.

Research conclusions

BrpR/BrpS are likely a new two-component system which regulates biofilm formation in *S. aureus*.

Research perspectives

A better understanding of a new regulator of *S. aureus* biofilm formation has been identified.

ACKNOWLEDGEMENTS

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FOOTNOTES

Author contributions: Zank A, Wescott A, and Schwan WR designed the research study; Zank A, Schulte L, Brandon X, Carstensen L, Wescott A, and Schwan WR performed the research; Zank A, Brandon X, and Schwan WR contributed new plasmids; Zank A, and Schwan WR analyzed the data and wrote the manuscript; and all authors have read and approved the final manuscript.

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Institutional review board statement: No humans or samples from human were used in this study.

Institutional animal care and use committee statement: No animals were used in this study.

Conflict-of-interest statement: Schwan WR holds a composition of matter and use patent covering the SK-03-92 Lead compound.

Data sharing statement: The authors will share their data with whomever asks.

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CASE REPORT

Pericarditis following COVID-19 vaccination: Two case reports

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Abstract

BACKGROUND

Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness which conventionally manifests with primarily respiratory symptoms and less commonly with cardiac involvement in various forms, such as pericarditis. Myocarditis and pericarditis have been reported in a variety of live and attenuated vaccines, such as smallpox and influenza. As of October 2021, no cases of pericarditis associated with COVID-19 vaccination have been published. We present two healthy male patients who present post COVID-19 vaccination with pericarditis diagnoses.

CASE SUMMARY

A 21-year-old male with no significant past medical history presented with myalgia, chills, mild headache, and chest pain for two days. Patient received the Moderna COVID-19 vaccine the day prior to symptom onset. On presentation, electrocardiogram (ECG) revealed sinus rhythm with ST elevation, and troponin was elevated. Emergent cardiac catheterization was not significant for abnormalities. The primary diagnosis was acute pericarditis, and the patient was discharged on colchicine and indomethacin. Additionally, a 35-year-old male with no pertinent past medical history presented with fever, chills, weakness, nausea, vomiting, diarrhea, and retrosternal chest pain for three days. He received the Moderna COVID-19 vaccine four days prior to symptom onset. On presentation, troponin was elevated, and ECG revealed mild ST elevation. Left ventricular dysfunction with ejection fraction of 41% was reported on transthoracic echocardiogram. Patient was started on ibuprofen and colchicine for diagnosis of

myopericarditis.

CONCLUSION
These case reports highlight a potential unintended consequence, pericarditis, associated with COVID-19 vaccination that may not warrant invasive cardiac intervention.

Key Words: Pericarditis; Myocarditis; COVID-19; COVID-19 vaccine; Myopericarditis; Case report

Core Tip: Coronavirus disease 2019 (COVID-19) manifests with primarily respiratory symptoms and less commonly with cardiac involvement. Myocarditis and pericarditis have been reported in a variety of live and attenuated vaccines. However, to our knowledge, there are no published cases associated with COVID-19 vaccination as of October 2021. We present two cases of pericarditis following COVID-19 vaccination. Both patients were treated with colchicine and non-steroidal anti-inflammatory agents but with varying degrees of invasive work-up. The first patient had emergent cardiac catheterization, while the second patient underwent computed tomographic angiography of the coronary arteries. Neither patient required intervention, thus questioning the necessity of cardiac catheterization.

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INTRODUCTION

On January 9, 2020, the World Health Organization announced a mysterious Coronavirus-related pneumonia in Wuhan, China[1]. Shortly after, cases were identified across the globe and quarantines were ordered to help prevent transmission. Despite early prevention efforts of social distancing and donning masks, coronavirus disease 2019 (COVID-19) was relentless[1]. As of November 2021, there are over 250 million confirmed cases and over five million deaths attributed to COVID-19[2]. The development of a vaccine was the light at the end of the tunnel but not without some associated risks.

Cardiac complications, such as myocarditis and pericarditis, after immunizations are extremely rare events in both younger and older patients but have been reported following smallpox, diphtheria, tetanus, polio, human papillomavirus (HPV), and influenza vaccines[3-6]. Through a PubMed search of pericarditis associated with the COVID-19 vaccine, no published cases have been identified. However, recently, the possible link between myocarditis and the COVID-19 vaccine has been under investigation by the Israel Health Ministry[7]. Sixty-two cases of myocarditis in young males were identified, but at this time, no conclusions have been made. The European Medicines Agency and United States Centers for Disease Control and Prevention (CDC) are also investigating the link between pericarditis and myocarditis with the COVID-19 vaccine, but no association has been found[8,9].

Here below, we present two cases of pericarditis in two young adult males following Moderna COVID-19 vaccination. A 21-year-old healthy male presenting three days post vaccination, and a 35-year-old male presenting seven days post vaccination. The purpose of this case report is to highlight an atypical response to the COVID-19 vaccine.

CASE PRESENTATION

Chief complaints

Case 1: In April 2021, a 21-year-old man was admitted to the hospital with myalgia, chills, mild headache, and chest pain for two days.

Case 2: In February 2021, a 35-year-old man presented to the emergency department with fever, chills, weakness, nausea, vomiting, diarrhea, and retrosternal chest pain for three days.

History of present illness

Case 1: Chest pain was rated three out of ten which worsened on inspiration and described as pleuritic in nature. The patient received the first dose of the Moderna COVID-19 vaccine series the day prior to

symptom onset. No associated diaphoresis or arm pain. No tenderness on palpation. No history of known COVID-19 infection or current fevers, nausea, vomiting, diarrhea, abdominal pain, shortness of breath, cough, extremity swelling, travel, or sick contacts.

Case 2: He received his first Moderna COVID-19 vaccine four days prior to symptom onset. Patient denies recent travel, sick contacts, COVID-19 exposure, or symptoms associated with COVID-19.

History of past illness

Case 1: The patient has no pertinent past medical history or surgeries.

Case 2: Past medical history significant for obesity, chronic allergic rhinitis, and previous COVID-19 infection reported several months prior to vaccination.

Personal and family history

No tobacco, illicit drugs, or alcohol use reported. No family history of heart disease for either of the two cases.

Physical examination

Case 1: In the emergency department (ED), on physical examination, no rub, normal rate, and regular rhythm noted.

Case 2: In the ED, on physical exam, the patient was noted to be tachycardic but otherwise had no abnormal physical exam findings, including pericardial rub.

Laboratory examinations

Case 1: Troponin was elevated at 15.2 ng/mL, creatinine kinase elevated at 657 units/L, C-Reactive Protein (CRP) elevated at 6.3 mg/dL, and erythrocyte sedi-mentation rate (ESR) mildly elevated at 24 mm/hr. Toxicology screen was negative. COVID-19, respiratory syncytial virus (RSV), and Influenza were negative using a sample obtained from a nasopharyngeal swab tested on Cepheid Xpert Xpress severe acute respiratory syndrome coronavirus 2/influenza/RSV reverse transcriptase polymerase chain reaction (SARS-CoV-2/Flu/RSV RT-PCR).

Case 2: Troponin was elevated at 7.58 ng/mL, CRP elevated at 26.8 mg/dL, and ESR elevated at 96 mm/hr. White blood cell (WBC) count was also elevated at 20.4 K/mcL. Bacterial and viral infectious etiologies were ruled out including COVID-19, RSV, influenza, bocavirus, adenovirus, parainfluenza, metapneumovirus, rhinovirus, enterovirus, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* using a sample from a nasopharyngeal swab tested on Cepheid Xpert Xpress SARS-CoV-2/Flu/RSV RT-PCR and Multiplex RT PCR amplification followed by Liquid Bead Array Hybridization. Hepatitis and human immunodeficiency viruses were also ruled out *via* antibody and antigen testing. Blood cultures had no growth.

Imaging examinations

Case 1: Chest X-ray was unremarkable with clear lungs and normal heart size. Transthoracic echocardiogram (TTE) demonstrated an ejection fraction (EF) of 65% without wall motion abnormalities and no pericardial effusion. Original electrocardiogram (ECG) reported sinus rhythm with ST elevation (Figure 1A). On repeat ECG, there was disproportionate worsening of ST elevation in inferior leads with development of new ST depression in aVL lead, so the patient was worked up for acute inferior myocardial infarction to rule out obstructive etiology. Emergent cardiac catheterization demonstrated 40% stenosis of the midsegment of the left anterior descending artery (LAD), though the remaining coronary arteries appeared to be disease-free and no interventions warranted.

Case 2: Chest X-ray and computed tomographic angiography (CTA) of the chest with contrast were both unremarkable. ECG demonstrated mild diffuse ST/T elevation (Figure 1B). On TTE, left ventricular dysfunction with EF of 41% reported.

FINAL DIAGNOSIS

Case 1: The primary diagnosis was acute pericarditis.

Case 2: The final diagnosis was myopericarditis complicated by acute kidney injury (AKI) secondary to intravenous (IV) contrast.

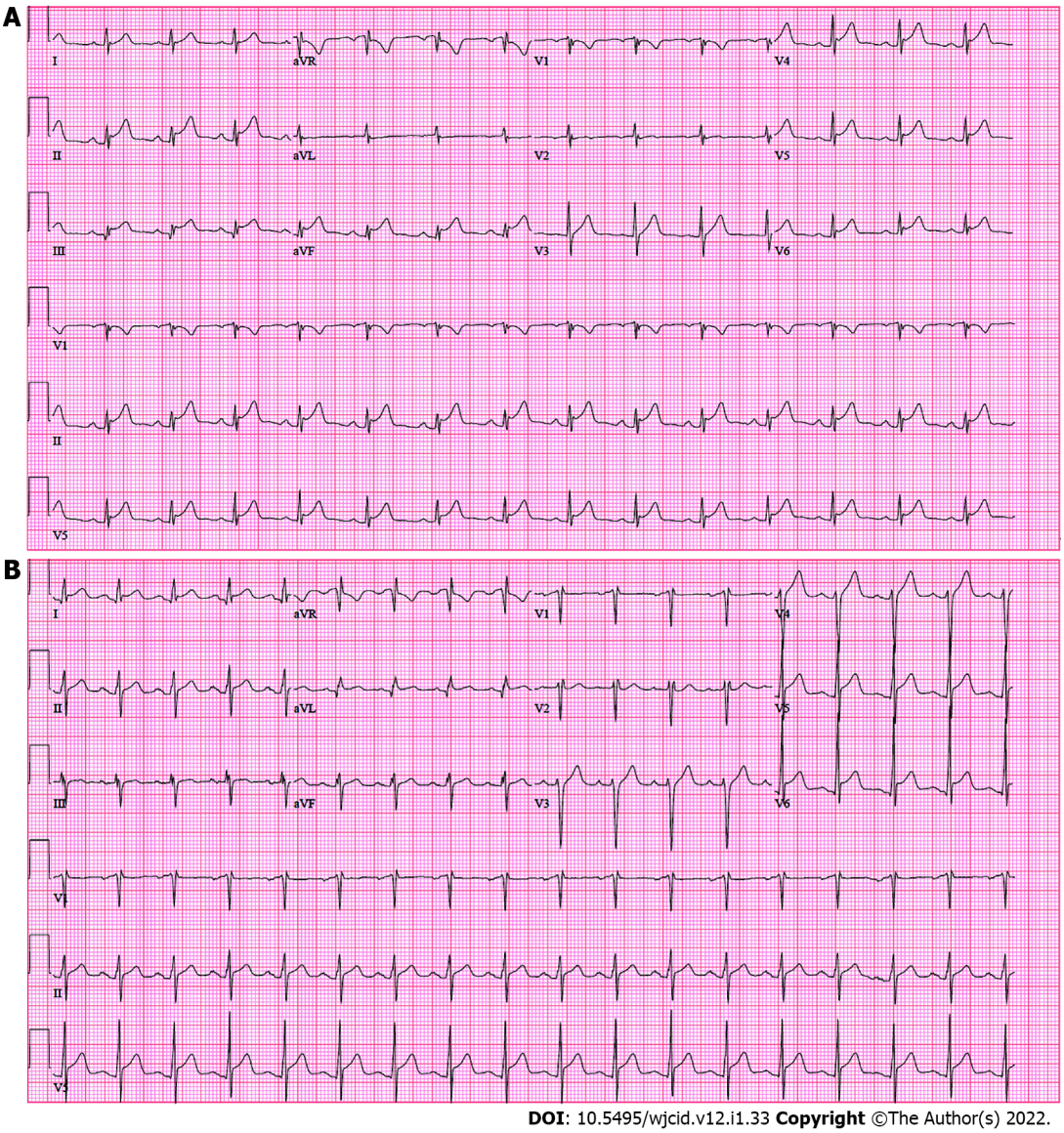

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Figure 1 Electrocardiogram of 21-year-old male (A) and 35-year-old male (B) showing ST elevation.

TREATMENT

Case 1: The patient was started on colchicine, indomethacin, and aspirin. He was discharged the following day on aspirin, colchicine for 14 d, and an indomethacin taper over 28 d.

Case 2: The patient was given acetaminophen and ketorolac in the ED. Cardiology was consulted and started the patient on ibuprofen and colchicine for myopericarditis. Ibuprofen was changed to prednisone due to contrast induced AKI. Colchicine and prednisone were continued for five additional days on discharge with instruction to follow-up with cardiology outpatient. Carvedilol and sacubitril/valsartan were initiated based on left ventricular dysfunction with plans to monitor and titrate outpatient. No antimicrobials were administered, and leukocytosis spontaneously resolved.

OUTCOME AND FOLLOW-UP

Case 1: Upon discharge, an appointment was scheduled with a new primary care provider with instruction to obtain a cardiologist referral. During follow-up with cardiology three months after discharge, repeat TTE was unremarkable with normal function and no regional wall motion abnormalities. Troponin, CRP, and ESR were normal as well. No further work-up or cardiology follow-up warranted. To our knowledge, there were no complications or readmissions associated with pericarditis following discharge.

Case 2: The patient's course was complicated by AKI secondary to IV contrast which extended his hospitalization to four days. Coronary CTA was later completed outpatient with no notable findings. To our knowledge, there were no complications or readmissions associated with pericarditis following discharge.

DISCUSSION

As of November 2021, over three billion individuals are fully vaccinated for COVID-19[10]. Common side effects of the COVID-19 vaccine are pain, redness, and swelling of the injection site, fatigue, headache, muscle pain, chills, fever, and nausea. No case reports of pericarditis as a side effect of COVID-19 vaccines have been published as of November 2021. A hypothesized mechanism of action of vaccine induced myopericarditis is an autoimmune reaction that typically occurs after seven days. Our patients presented within seven days post vaccination; therefore, this mechanism is unlikely[11]. A possible mechanism for male predominance in myocarditis/pericarditis is presumably related to variations in sex hormones. Estrogen is thought to inhibit proinflammatory T cells, resulting in a decrease in immune related responses. Pericarditis in females may also be underdiagnosed, which could explain the male predominance[12].

On the contrary, active COVID-19 infection presenting as pericarditis and endocarditis have been reported[13,14]. A systematic review conducted in December 2020 identified 34 COVID-19 patients with pericarditis reported, and 62% were diagnosed with myopericarditis[15]. Pericarditis in COVID-19 patients is still poorly understood. A proposed explanation is the binding of the SARS-CoV-2 Spike protein to angiotensin-converting enzyme 2 which can be found on the heart. Thus, there is potential cause for myocarditis, pericardial effusion, and pericarditis[13,15].

While no case reports were published to our knowledge, as of May 2021, 133 cases of pericarditis and 119 cases of myocarditis had been reported to the CDC Vaccine Adverse Event Reporting System (VAERS)[16]. Cases of pericarditis were reported following each of the Food and Drug Administration approved COVID-19 vaccines Pfizer (67), Moderna (54), and Johnson and Johnson (12). Pfizer was the first vaccine to be approved as well as the most common manufacturer associated with reported pericarditis cases, followed by Moderna, then Johnson and Johnson. Of the 133 pericarditis cases, 77 reported cases are males, 53 are females, and 3 were not reported. The average age for males was 44.6 years old and ranged from 16 to 84 years old. The average age for females was 54.5 years old and ranged from 20 to 85 years old. The mean ages are likely falsely elevated based on vaccine rollout and earlier access to patients 65 years and older in the general public. The average reported time to symptom onset is 7.4 d and ranges from 0 to 63 d. Our patient cases consist of two males that are below the mean reported age and below the average time to symptom onset. As of November 2021, the number of adverse events with the word "pericarditis" associated with the COVID-19 vaccine has since substantially increased to 15895 reports[16].

Acute pericarditis is most commonly caused by viral infections and has been reported in patients with active COVID-19 infections[14,17-19]. Our case report is unique because we present two individuals diagnosed with pericarditis post COVID-19 vaccination who tested negative for active COVID-19 infection. Pericarditis is diagnosed when at least two of the four following criteria are present: Pericarditic chest pain, pericardial rubs, new widespread ST elevation or PR depression on ECG, or pericardial effusion[20]. Additional supporting findings include elevation of cardiac inflammatory markers and evidence of pericardial inflammation by imaging[20]. Both of our patients presented with ST elevation, pericarditic chest pain, along with several elevated cardiac inflammatory markers. In the second case, ECG does not show the typical patterns of acute pericarditis (*i.e.* diffuse ST elevations with PR depressions), which are often seen hours to days after symptom onset during the first stage. Considering the patient presented seven days post-vaccination and three days after symptom onset, it is plausible any atypical ECG patterns had since normalized. Neither patient had diagnosed cardiac history, infectious causes, noninfectious causes, or past medical history to predispose them to pericarditis; therefore, we believe these episodes of acute pericarditis are secondary to the COVID-19 vaccine.

Patients presenting with COVID-19 and elevated troponins often have worse outcomes[21]. However, if patients present following COVID-19 immunization with elevated cardiac inflammatory markers, ST elevation, but not diagnosed with COVID-19, should cardiac catheterization be standard of care? The 21-year-old patient was taken for emergent cardiac catheterization and was found to have 40% stenosis of the midsegment of the LAD, but remaining coronary arteries appeared to be disease-free. No lesions were identified, and no interventions warranted. While no complications occurred with this patient, possible complications of invasive cardiac catheterization include infection, vascular complications, bleeding, stroke, myocardial infarction, and death[22]. The 35-year-old patient did not undergo cardiac catheterization but did receive a CTA of the chest and coronary arteries instead, which was unremarkable. It is unknown if the pericarditis cases reported to CDC VAERS warranted catheterization or other invasive testing. But of the 133 individuals reported, 86 were hospitalized, 46 were not hospitalized, and one unknown, which leads us to believe many cases self-resolved without seeking out

higher level of care.

Our patient cases do have inherent limitations. In the second case, since the patient reported a previous diagnosis of COVID-19, it is impossible to completely rule out myopericarditis as a complication from previous infection. The patient tested negative for active infection on admission, but past COVID-19 test results could not be found in the electronic medical record, so an exact date of infection is unknown. To our knowledge, neither patient has received the second immunization in the series; therefore, we are unable to assess outcomes when re-challenged with the second dose. Additionally, due to a lack of endomyocardial biopsy, a histopathological diagnosis for pericarditis or myocarditis cannot be confirmed. Both patients presented to a community hospital where there was not access to cardiac MRI, and based on rapid clinical improvement, myocardial biopsy was not warranted. Furthermore, other causes of pericarditis cannot be completely ruled out. The first patient tested negative for COVID-19, influenza, and RSV. Other infectious causes are less likely but were not tested for. COVID-19, RSV, influenza, bocavirus, adenovirus, parainfluenza, metapneumovirus, rhinovirus, enterovirus, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae* were ruled out for the second patient, making viral and bacterial causes unlikely. Neither of these patients have a significant past medical history, including no likely medication causes, no trauma, nor autoimmune conditions. We also do not have long-term follow-up, thus no long-term outcomes for either patient. Despite these limitations, both patients scored a 5 on the Naranjo Algorithm, or Adverse Drug Reaction Probability Scale, resulting in a probable association. The reaction "followed a reasonable temporal sequence after a drug, followed a recognized response to the suspected drug, was confirmed by withdrawal but not by exposure to the drug, and could not be reasonably explained by the known characteristics of the patient's clinical state." [23].

During the time of these cases, the new mRNA COVID-19 vaccinations were under emergency use authorization. It is important to note, despite the skepticism and fear surrounding these novel vaccines, the benefits greatly outweigh the risk of rare side effects, including pericarditis and myocarditis. In fact, the CDC has reported the rare incidence of myocarditis/pericarditis as about 12.6 cases per million doses of second-dose mRNA vaccines in those age 12 to 39 years old (0.0000126%)[12]. However, a study investigating cardiovascular sequelae in COVID-19 infected patients revealed that 5.0% developed new-onset myocarditis and 1.5% developed pericarditis; therefore, there is much higher risk from active infection than vaccination[24]. The COVID-19 mRNA vaccines reduce the rate of severe infections, hospitalizations, and death from COVID-19. In a study conducted at five Veteran Affairs Medical Centers, mRNA vaccines were 86.8% effective at preventing COVID-19 associated hospitalizations in those who were over the age of 18 years old[25]. Most episodes of pericarditis are uncomplicated and can be managed in the outpatient setting[18]. Therefore, these cases should alleviate the fear of vaccine associated adverse effects and help guide the public on when to seek care.

CONCLUSION

Despite our findings and presumed correlation of COVID-19 vaccination and pericarditis, this should not deter individuals from being vaccinated, especially given the reported cardiac involvement from COVID-19 infections. Immunizations are essential for public health and achieving population immunity. Rather, these cases are intended to bring awareness to a potential etiology of pericarditis that should be considered in the differential that might not warrant invasive interventions with substantial risks. Further research and trials are needed to assess the linkage between COVID-19 vaccination and cardiac injury. Our cases highlight the importance of recognizing the possibility of COVID-19 vaccine side effects presenting as pericardial injury in young otherwise healthy individuals. The question remains, is cardiac catheterization necessary for every patient who presents with pericarditis secondary to COVID-19 vaccination?

FOOTNOTES

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CASE REPORT

Unusual cause of hemorrhagic pleural effusion: A case report

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Abstract

BACKGROUND

Infected aortic aneurysms are uncommon and difficult to treat. We present a case of infected aortic aneurysm with recurrent nontyphoidal *Salmonella* bacteremia.

CASE SUMMARY

A 68-year-old gentleman presented with non-specific symptoms and was found to have nontyphoidal *Salmonella* bacteremia and was treated with intravenous ceftriaxone. However his condition did not improve, and he developed a multiloculated right pleural effusion. Thoracocentesis was done to drain hemorrhagic pleural fluid. Chest computed tomography demonstrated descending thoracic aorta saccular aneurysm with periaortic hematoma likely due to recent bleed and extending to the right pleural cavity. He was referred to cardiothoracic surgery team and was planned for medical therapy in view of hemodynamic stability and no evidence of active leakage. He completed intravenous antibiotic for 5 wk and refused surgical intervention. Unfortunately, he was admitted twice for recurrent nontyphoidal *Salmonella* bacteremia. Finally, he agreed for surgical intervention and underwent endovascular aortic repair 3 mo later. Postoperatively, his condition remained stable with no recurrence of infection.

CONCLUSION

Our case highlights the importance of high index of suspicion of infected aortic aneurysm in patients with *Salmonella* bacteremia with high-risk factors such as atherosclerosis.

Key Words: Infected aneurysm; Aorta; Nontyphoidal *Salmonella*; Pleural effusion; Case report

Core Tip: Infected aortic aneurysm is a rare condition with high mortality. Our aim of this case report is to highlight the importance of high index of suspicion of infected aortic aneurysm in patients with *Salmonella* bacteremia with additional literature review to help clinician in the management of this disease. Medical therapy alone in this condition is associated with poor outcome.

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INTRODUCTION

Infected aneurysm is a rare disease associated with significant morbidity and mortality. Initial symptoms are non-specific, and due to lack of conclusive signs and symptoms, patients are often subjected to various investigations until a diagnosis is made. Standard treatment consists of antibiotic therapy and open surgery with surgical debridement and vascular reconstruction. However, there are no clear guidelines or randomized controlled trial on the best approach for the management of this condition. We report a patient with nontyphoidal *Salmonella* infected aortic aneurysm who was treated with medical therapy initially and complicated with recurrence. He was successfully treated with endovascular aortic repair and was well during follow-up with no recurrence of infection. Relevant literature is reviewed.

CASE PRESENTATION

Chief complaints

A 68-year-old man presented to our hospital with fever associated with lethargy, reduced oral intake, and dyspnea.

History of present illness

The patient's presenting symptoms had lasted for 1 wk.

History of past illness

His medical illness includes diabetes mellitus, hypertension, ischemic heart disease, and chronic kidney disease.

Personal and family history

No relevant family history.

Physical examination

He was febrile and required the support of face mask oxygen during presentation. Examination revealed reduced air entry over right lower zone with bilateral lower zone crepitations.

Laboratory examinations

Laboratory results showed white blood cell count of 16 × 10^9/L, hemoglobin level of 8.3 g/dL, urea 16.9 mmol/L, and creatinine 301 μmol/L.

Imaging examinations

Chest X-ray on admission showed blunted right costophrenic angle (Figure 1).

PROGRESS

He was admitted to the medical ward with the diagnosis of pneumonia and acute on chronic kidney disease. He was started on intravenous (IV) ceftriaxone 2 g daily. Blood culture on admission was positive for nontyphoidal *Salmonella spp*. During the course of admission, he became more tachypneic, and right thoracocentesis was done in view of worsening pleural effusion, which drained out 250 mL of blood stained fluid. Lung ultrasound showed multiseptated right pleural effusion. Despite ultrasound guided pigtail drainage of right pleural effusion, his condition did not improve. Chest computed

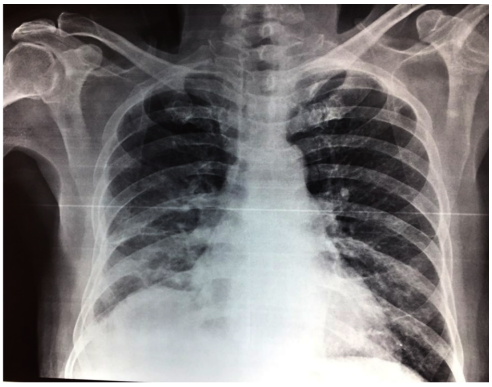

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Figure 1 Chest X-ray on admission showed right pleural effusion.

tomography (CT) demonstrated descending thoracic aorta saccular aneurysm with periaortic hematoma (Figure 2), likely due to recent bleed, and extending to the right pleural cavity. Pleural fluid culture was negative.

FINAL DIAGNOSIS

Salmonella infected aortic aneurysm.

TREATMENT

He was referred to cardiothoracic surgery team and was planned for medical therapy in view of hemodynamical stability and no evidence of active leakage. There were multiple changes in his antibiotics regimen due to persistent fever (which included ceftazidime, piperacillin-tazobactam, meropenem, and cefepime). He completed 5 wk of antibiotics and was planned for a CT angiography of aorta at a later date to decide on surgical intervention.

OUTCOME AND FOLLOW-UP

Unfortunately, the patient was readmitted 2 mo later with abdominal discomfort and unable to pass motion for 1 wk. Abdominal X-ray demonstrated dilated large intestine. CT abdomen and pelvis showed descending thoracic aorta saccular aneurysm (slightly larger) with features suggestive of superimposed infection of periaortic hematoma; size of aneurysm: 2.4 cm × 3.6 cm and fecal laden bowels with features of impending intestinal obstruction. No obvious bowel related mass was seen. He was treated conservatively for the ileus, which resolved after 1 d. Blood culture on this admission was positive again for nontyphoidal *Salmonella spp*. Echocardiogram did not show any vegetations. He completed 6 wk of IV ampicillin and was discharged well. A repeated CT scan after 2 mo showed resolved periaortic hematoma; however, the patient refused surgical intervention and was given lifelong prophylactic oral antibiotic. Unfortunately, patient presented again with second recurrence after 1 mo, and blood culture was positive again for non-typhoidal *Salmonella*. He completed IV antibiotic and finally agreed for surgical intervention. He underwent endovascular aortic repair, and his condition remained stable with no recurrence of infection during his last follow-up after 2 years.

DISCUSSION

Infected aneurysm is a serious clinical condition. The term mycotic aneurysm was first described by William Osler in 1885[1], however the nomenclature of mycotic aneurysm *vs* infected aneurysm remains controversial as a majority of infected aneurysms are due to bacterial infection. Etiology of infected aneurysm includes direct bacterial inoculation, bacteremic seeding of existing intimal injury, atherosclerotic plaque, or preexisting aneurysm, contiguous infection, or septic emboli from heart, which can occlude vasa vasorum of blood vessel and lead to infected aneurysm.

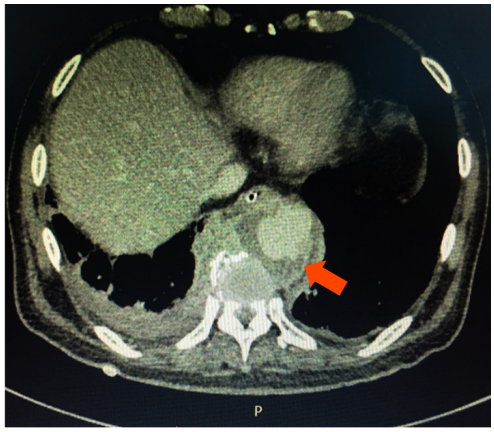

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Figure 2 Chest computed tomography showed descending thoracic saccular aneurysm with periaortic hematoma (arrow).

Infected aortic aneurysm is a rare but life-threatening condition with devastating outcomes. Initial clinical presentations are often non-specific; therefore, misdiagnosis is common. Infected aortic aneurysms may manifest as vague pain at the back, chest, or abdomen. Some patients may present as pyrexia of unknown origin and remain undiagnosed until rupture of aneurysm. In our patient, he was only diagnosed with infected aortic aneurysm after 3 wk of admission.

Salmonella species and *Staphylococcus aureus* are the most common pathogens of infected aortic aneurysm, followed by other organisms such as *Streptococci* species, *Treponema pallidum*, and *Mycobacterium spp*[2-4]. In East Asia, gram negative bacteria related infected aortic aneurysm is more prevalent, where *Salmonella* species are the most common organisms[2,3,5,6]. It is important to obtain a microbiological diagnosis given the need for protracted courses of antimicrobial therapy. However, blood cultures are negative in around 50% of cases[5,7,8]. Initial empirical treatment is often required and guided by the most likely infecting organism according to the individual and clinical circumstances. It is also prudent to use antibiotics judiciously as prolonged broad spectrum antibiotics may lead to development of antimicrobial resistance. *Salmonella* has a strong affinity for large blood vessels and can easily adhere to the damaged vascular wall, strongly affecting the natural course of the disease. However, studies showed that non-*Salmonella* infections are associated with higher aneurysm-related complications and mortality after treatment[9].

The study by Oderich *et al*[10], with 43 patients with infected aortic aneurysm, found that the risk factors of aneurysm-related death were extensive periaortic infection, female gender, *Staphylococcus aureus* infection, aneurysm rupture, and suprarenal aneurysm location. Another larger series done in Taiwan reported that the independent predictors of aneurysm-related death were advanced age, non-*Salmonella* infection, and non-surgical treatment[2].

Until now, there are no randomized clinical trials to guide the management of infected aneurysm. Therapy mainly includes the control of infections by antibiotic and surgical debridement with or without reconstruction of arterial circulation. Management strategies are primarily based upon clinical experience guided by case series. The optimal duration of antibiotic therapy remains inconclusive. Most studies recommend at least 6 wk of antibiotic and stopping only when there is no longer clinical and laboratory evidence of ongoing sepsis[11,12].

Medical therapy alone is associated with devastating outcomes, with in-hospital mortality of 50% [13]. Since infected aortic aneurysm is at high risk of rupture, surgical treatment is encouraged in the absence of absolute contraindications. Open surgery with extensive debridement of infected tissue and *in situ* or extra-anatomical reconstruction has been the gold standard treatment. However, it is associated with high risk of morbidity and mortality[3,14-16]. Recently, endovascular techniques has become an emerging treatment alternative, especially in the treatment of patients at prohibitive risk for open surgery. Few studies have shown that endovascular treatment of infected aortic aneurysm is feasible and that it is a durable treatment option for high risk patients[17,18]. Luo *et al*[18] reported that survival at 1 mo, 6 mo, 1 year, and 5 year was 90%, 82%, 71%, and 53%, respectively. In addition, a European multicenter study of endovascular treatment for infected aortic aneurysm also showed similar results[17].

CONCLUSION

Infected aortic aneurysm is a rare clinical entity with high mortality. Due to non-specificity of the early symptoms, misdiagnosis is common. Our case highlights the importance of high index of suspicion of

infected aortic aneurysm in patient with recurrent nontyphoidal *Salmonella* bacteremia. Endovascular technique has become an emerging treatment option.

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FOOTNOTES

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LETTER TO THE EDITOR

COVID-19, stigma, and people with disabilities: A mental health perspective

Raktim Swarnakar, Shreya Santra

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Abstract

Discrimination is an age-old 'illness' irrespective of its context. Stigma is a common factor that has been associated with disability and coronavirus disease 2019. The public health impact of stigma on differently-abled people during this pandemic is not known and it is a poorly investigated and neglected area. It is important to address the current research need in the concerned area and its implications for public health policymaking and changes in practices that it requires. Together we can win the war against pandemics if we reduce the mental distancing in all perspectives.

Key Words: COVID-19; Stigma; Disability; Mental health; Public health

Core Tip: Currently, coronavirus disease 2019 (COVID-19) is known to be associated with stigma. Previously, it was known that disability is also associated with stigma. The public health impact of stigma on differently-abled people during the COVID-19 pandemic is not known and is a poorly investigated area currently. This letter would like to address the current research need in the concerned area and this would have implications for public health policymaking and changes in practices that it needs.

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TO THE EDITOR

"Viruses do not discriminate and neither should we"[1].

Globally, coronavirus disease 2019 (COVID-19) has become a public health emergency. In such crisis, rumors, misinformation, fear, and lack of proper public health awareness make fertile soil for the stigma to grow incessantly[2]. Unfortunately, from historical ages to the modern era, infectious diseases and disabilities are independently associated with the social stigma. COVID-19 has already made a negative impact on mental health and stigma has just aggravated it. It is well known that people with disabilities face discrimination and stigma in different spheres of life and such a pandemic situation resulted in greater difficulty in individuals with disabilities than the able-bodied population.

Stigma invariably leads to concealment of COVID-19 symptoms and delayed treatment, which leads to greater dissemination of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection among the public. Many disabling conditions like people with spinal cord injury are particularly vulnerable to SARS-CoV-2 infection. People with disabilities already had physical barriers[3], but the pandemic has added mental and attitudinal barriers due to social stigma. In the context of pandemic crisis, such social stigma severely affects the mental health of people with disabilities. COVID-19 already has hampered social participation due to social distancing and has limited functional involvement due to home confinement and lockdown[4]. Moreover, vaccine inequity may also create further issues which need attention beforehand[5]. Furthermore, social stigma becomes an extra hindrance for better functionality and participation of these populations.

Social stigma in the context of mental health not only damages its victim but becomes also detrimental to the whole public health domain. Social stigma among individuals with disabilities during a pandemic can be prevented by: (1) Public health awareness program through proper information, education, and communication; (2) breaking the misconceptions about COVID-19; (3) considering people with disabilities as differently-abled; (4) improving provision of telerehabilitation emphasizing psychiatric telerehabilitation during the pandemic; (5) identifying barriers and planning to overcome them; and (6) online social-engagement, peer-group formation, and motivational sessions to boost morale and improve the mental well-being of individuals with special needs and disabilities.

Worldwide COVID-19 cases crossed 336 million, and the population with disability crossed one billion[6,7]. Putting this situation in the public and mental health perspective, COVID-19 has set a new-normal life whereas people with disabilities lead a new-normal life with different ability, and eradicating social stigma from this 'new-normal life' is each and everyone's responsibility.

We, healthcare professionals from every domain, should keep closer surveillance so that the physical distancing does not become a mental distancing.

What is the current understanding of this topic?

Currently, COVID-19 is known to be associated with stigma. Previously, it was known that disability is also associated with stigma.

What does this Letter-to-Editor add to the literature?

The public health impact of stigma on differently-abled people during the COVID-19 pandemic is not known and is a poorly investigated area currently.

What are the implications for mental health practice?

This letter would address the current research need in the concerned area and this would have implications for public health policymaking and changes in practices that it needs.

FOOTNOTES

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WJCID mainly publishes articles reporting research results and findings obtained in the field of infectious diseases and covering a wide range of topics including community-acquired infections, cross infection, eye infections, focal infection, infectious gingivitis, intraabdominal infections, laboratory infection, Ludwig's angina, necrotizing ulcerative periodontitis, opportunistic infections, pelvic infection, pregnancy complications, *etc*.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Clinical significance of anti-nucleocapsid-IgG sero-positivity in SARS-CoV-2 infection in hospitalized patients in North Dakota

Bakir Dzananovic, Mark Williamson, Casmiar Nwaigwe, Chittaranjan Routray

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Abstract

BACKGROUND

During the peak of the coronavirus diseases 2019 (COVID-19) pandemic, clinicians actively studied the utility of various epidemiologic-clinical parameters to determine the prognosis for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. Serum IgG antibody level, D-Dimer, C-reactive protein and neutrophil to lymphocyte ratio, *etc.* were studied to assess their association with the clinical course in hospitalized patients and predict who may be at increased risk for poor clinical outcome. However, the influence of SARS-CoV-2-anti-nucleocapsid-IgG antibody (IgG-N) sero-positivity on the clinical outcome of patients with COVID-19 is largely unknown.

AIM

To study the influence of SARS-CoV-2 anti-nucleocapsid-IgG seropositivity on clinical course and diseases severity in hospitalized COVID-19 patients.

METHODS

We conducted a retrospective study of adults admitted to a tertiary care community hospital in North Dakota with COVID-19. Included patients had severe

COVID-19 disease or worse and so required supplemental oxygen on admission. They were serologically tested for SARS-CoV-2-anti-nuceocapsid-IgG (IgG-N). The IgG-N positive group were 26 patients and the IgG-N negative group had 33 patients. The groups received similar treatment for COVID-19 as approved by our healthcare system from Day 1 of admission until discharge or death. Measurable parameters for monitoring the patients' clinical course included the following: Length of hospitalization (LOS), use of high flow nasal canula (HFNC), use of noninvasive bilevel positive pressure ventilation (BiPAP), admission into the intensive care unit, need for mechanical ventilation (VENT); and the patient outcome/discharge or death. Other variables included were age, gender and body-mass-index, and duration of symptoms before presentation. For each variable, the outcome was modeled as a function of SARS-CoV-2-IgG-N status (positive or negative) using a generalized linear model. For LOS-days, a negative binomial distribution was used as it had a better fit than a Poisson or Gaussian distribution as evidenced by a Pearson chi-square/df value closer to 1.0. All other outcomes utilized a binary logistic regression model.

RESULTS

After a thorough examination of patient data, it was found that admission rates to the Intensive Care Unit, as well as the usage of BiPAP, HFNC and VENT support, in conjunction with patient outcomes, were not significantly different across IgG-N status. However, the LOS variable when assessed by IgG-N status was found to be significant (t value = 2.16, P value = 0.0349). IgG-N negative patients had higher than average LOS in comparison to IgG-N positive patients (15.12 *vs* 9.35 d). Even when removing the extreme value (an LOS of 158 d), IgG-N negative patients still had slightly higher than average stays (10.66 *vs* 9.35 d) but the relationship was no longer significant. For patient outcome/death, only age (numerical) was a significant predictor (F value = 4.66, P value = 0.0352). No other variables for any of the outcomes were significant predictors of clinical course or disease severity.

CONCLUSION

Our study demonstrated that IgG-N seroconversion had no significant association with clinical outcomes in hospitalized COVID-19 patients.

Key Words: COVID-19; SARS-CoV-2 IgG-N; Anti-nucleocapsid IgG; Cytokines

Core Tip: We intended to study an immunologic marker to predict the need for advanced oxygen supplement system and clinical outcome in order to support our hospital crisis management system during the peak of the pandemic. Our study demonstrated that presence of anti-nucleocapsid-IgG (IgG-N) against severe acute respiratory syndrome coronavirus 2 infection had no impact on the clinical outcome or disease severity in hospitalized coronavirus disease 2019 (COVID-19) patients. We did not find a correlation of statistical significance to use IgG-N as a biomarker to predict clinical outcome in COVID-19 patients admitted to a community hospital in North Dakota.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus which belongs to the family of *Coronaviridae*, the causative agent for coronavirus diseases 2019 (COVID-19)[1]. SARS-CoV-2 emerged out of Wuhan in China and soon after, it spread to the entire world and thereby becoming a "Pandemic"[2-4]. After two years of rapid spread and the virus claiming over five million lives, healthcare system continues to scramble to protect patients from the atypical pneumonia-like illness caused by COVID-19. Diagnosis of SARS-CoV-2 infection is primarily dependent on reverse-transcription polymerase chain reaction (RT-PCR) testing of nasopharyngeal swab samples with more recent progress into rapid antigen testing[5,6]. Rapid community spread of the infection and dev-

elopment of herd immunity by community exposure has been a favorite topic of discussion by epidemiologists while the scientific community have successfully raced to design several effective vaccines for COVID-19.

Taking a dive into the pathophysiology of COVID-19, a strong comprehension of the role of the humoral immunity becomes very pertinent. It is known that infection with SARS-CoV-2, elicits an adaptive immune response by producing target specific antibodies which includes IgM, IgG and IgA[7-9]. Among these antibodies, IgG has been of tremendous interest to the scientific community due to its role in long-term protection against the virus[10]. After an infection with SARS-CoV-2, it takes about 10-14 d to produce IgG antibodies which peak around the third week and continues to remain detectable for about 8-12 mo[11,12]. SARS-CoV-2 is a positive sense single stranded RNA virus comprised of four different structural proteins. Those are the nucleocapsid protein (N protein), spike protein (S-protein), matrix protein (M-protein) and the envelope protein (E-protein)[13]. Antibody against the S-protein (IgG-S) is believed to be the neutralizing antibody that is the primary target of the vaccine trials. There have been reports showing a positive correlation between higher IgG-S levels and diseases severity[14, 15]. On the contrary, another study cited no association of IgG-S with patient outcomes such as need for maximal oxygen support, intensive care unit (ICU) admission, duration of hospitalization and death [16]. Alternatively, it could be argued that the non-neutralizing antibodies against the nucleocapsid protein (IgG-N) leads to robust inflammation cascade and release of cytokines thus contributing to debilitating pulmonary injury. It is believed that the cytokine storm plays a key role in the pathogenesis and COVID-19 prognosis[17,18]. A report by Batra *et al*[19] studied the role of IgG-N in COVID-19 and based on the findings the authors recommended using IgG-N titers as a prognostic factor for the clinical course in patients. In this study, a higher IgG-N titer was associated with extended duration of stay in the hospital and increased rate of admission into the ICU. Another study demonstrated that the stronger IgG-N seroconversion response is associated with more diseases severity compared to the weak responders[20]. There is still a paucity of data about the functionality of IgG-N in the pathophysiology of COVID-19. Given this concept of targeting various structural components of this prolific virus, study of the seroconversion and IgG-kinetics has gained a lot of importance to the researchers. Literature on the long-term kinetics of IgG antibody levels and their corresponding neutralizing effectiveness is sparse.

Practicing in a tertiary care community-based teaching hospital in North Dakota, United States, we have had experience with the pre-vaccine phase of COVID-19 pandemic. Noncompliance with public mask usage and rapid community transmission led to a sharp rise in COVID-19 illness and hospitalizations in North Dakota. In the midst of a healthcare crisis, we decided to investigate whether a qualitative IgG-N could be used as a molecular marker to determine the prognosis in the hospitalized patients. Basing our hypothesis on a theory that a rampaging community spread of SARS-CoV-2 infection led to a measurable IgG-N seroconversion of our population, thus impacting outcomes from hospitalization due to COVID-19, we retrospectively analyzed the data provided by the single-center community hospital from which we practiced.

MATERIALS AND METHODS

Study population and data collection

All patients were admitted to the community hospital between December 1, 2020 and August 30, 2021. Fifty-nine patients were included in the study who were screened for IgG-N within 48 h of admission. We excluded all patients that had been admitted to the hospital with a non-COVID-19 diagnosis who incidentally tested positive for SARS-CoV-2 during screening. Patients with severe or critical COVID-19 illness as per the definition of National Institute of Health were included in the study. Those with mild and moderate illness were excluded from the study as most of them did not meet criteria for hospitalization. None of the included patients had been vaccinated against COVID-19. All the patients were confirmed positive for SARS-CoV-2 infection using RT-PCR from nasopharyngeal swab samples at admission. Both male and female patients aged 28 to96 were included in the study. All patients were checked for the presence of SARS-CoV-2-IgG-N within 48 h of admission by using Abbott SARS-CoV-2-IgG assay, that uses a two-step chemiluminescent microparticle immunoassay method with acridinium-labeled anti-human IgG, performed at North Dakota state laboratory. Admission blood samples identified 26 patients positive for IgG-N against SARS-CoV-2 and 33 negatives. In October 2021, we started data acquisition, reviewing the electronic medical record of included patients.

Study design

As this retrospective cohort study investigated the study population from patient admission to outcome, a thorough review of the electronic medical record was performed to capture data. This data was inclusive of the following: age, gender, body mass index (BMI), duration of symptoms prior to hospitalizations (DOS), length of hospital stay measured in days (LOS), admission to ICU, need for high flow nasal cannula (HFNC), bilevel positive airway pressure ventilation (BiPAP) or mechanical ventilation (VENT) for supplemental oxygen/support, as well as the final patient outcome - discharge or death.

(Table 1)

Statistical analysis

Formatting: Age, BMI and DOS were numerical variables. However, additional constructs split Age and BMI into two and three-group categories for some analyses. For example, BMI_2, patients with a BMI of < 29.9 were put in one group, and those > 30 in a second group. For BMI_3, patients with a BMI of < 25 were put into one group, those between BMI of 25-29.9 in a second group, and those with BMI > 30 in a third. For the variable labelled Age_2, patients < 75 were put in one group, and those 75+ in a second group. For Age_3, patients with an age of < 40 were put into the first group, those between 40-75 in a second, and those 75+ in a third group. It should be noted here that one patient had an extreme value for their LOS at-158 d. Models were run with both the patient included and excluded to determine the sensitivity of the models to this extreme value.

Correlation of outcomes: For each pair of outcomes (Death, ICU, BiPAP, VENT and HFNC), the phi coefficient (measure of association between binary variable, comparable to the Pearson coefficient for continuous normal variables) was calculated (Table 2).

Outcomes by IgG status alone: For each variable, the outcome was modeled as a function of IgG-N status (positive or negative) using a generalized linear distribution. For LOS, it was determined that a negative binomial distribution had a better fit than a Poisson or Gaussian distribution, as evidence by a Pearson chi-square/df value closer to 1.0. The negative binomial distribution is also less sensitive to outliers. All other outcomes utilized a binary logistic regression model.

Outcomes by IgG-N status full model: For any single models that were significant, a multiple regression model was utilized, accounting for the consequential effect (if any) of the defined confounding variables of age, sex, BMI, and duration of symptoms.

Outcomes by other factors

LOS was modeled as a function of age using a negative binomial model. From there, age (categorical), BMI (numerical), and the interaction of BMI and age were each run with and without the extreme patient LOS-value noted in the previous section. The patient outcome/death was modeled as a function of LOS using a logistic model. Then, death was modeled as a function of age (numerical), and then age (categorical). The same was done for BMI. Finally, death was modeled as a function of sex. ICU, BiPAP, VENT, and HFNC were each modeled as a function of age (numerical), BMI (numerical), and sex separately.

Statistical analysis used SAS Studio V.3.8 (Cary, North Carolina, United States). The statistical review of the study was performed by a biomedical statistician.

RESULTS

We conducted a retrospective cohort study among fifty-nine adults aged between 28-96, admitted to the hospital with severe or critical COVID-19 illness between December 2020 and August 2021.

Correlation of outcomes

Unsurprisingly, most outcomes were strongly correlated. VENT and ICU rates were very strongly correlated (Phi Coeff = 0.94). All but one patient who went on mechanical ventilation was also admitted to the ICU. In contrast, VENT and HFNC rates were only moderately correlated (Phi Coeff = 0.43).

Outcomes by IgG status alone

Patient outcome, ICU admissions, HFNC, BiPAP and VENT rates were not significantly different across IgG-N status (Figure 1A-E). However, LOS by IgG-N status was found to be significant (t value = 2.16, P value = 0.0349) when including the extreme value (LOS > 150 d). IgG-N negative patients had higher average LOS than IgG-N positive patients (15.12 *vs* 9.35 d). However, when removing the extreme value (LOS of 150 d), IgG-N negative patients still had slightly higher average LOS (10.66 *vs* 9.35 d), but the relationship was no longer significant (Figure 1F). Furthermore, median LOS was lower in IgG-N negative patients (6.5 *vs* 7.5 d).

Outcomes by IgG-N status full model

Because LOS-days and IgG-N status was significant, at least when not removing the extreme value, the full model was considered which included age, BMI, and sex, and duration of symptoms. However, in the full model, IgG-N was not significant when controlling for the other variables. This remained true when using a model without the extreme value.

Table 1 Summary statistics by IgG-N status

	IgG-N positive	IgG-N negative
Demographic information		
Number	26 (19 male, 7 female)	32 (20 male, 12 female)
Mean age	61.1 (17.6)	60.0 (16.1)
Median age	63.5 (25)	63.5 (21)
Mean BMI	33.7 (7.4)	33.9 (7.7)
Median BMI	32.8 (12)	33.9 (10.1)
Mean DOS-d	7.5 (5.6)	6.5 (4.1)
Median DOS-d	5.5 (6.0)	7.0 (7.0)
Outcome		
Mean LOS-d	9.3 (5.6)	10.7 (10.4)
Median LOS-d	7.5 (7)	6.5 (7)
Proportion of death	0.23	0.19
Proportion of ICU	0.19	0.16
Proportion of BiPAP	0.27	0.22
Proportion of VENT	0.15	0.16
Proportion of HFNC	0.38	0.25

Standard deviation and Interquartile Range are found in parentheses for mean and medians respectively. DOS: Duration of symptoms (days); LOS: length of stay (days); ICU: Intensive care unit; BiPAP: Bilevel positive airway pressure ventilation; VENT: Mechanical ventilation; HFNC: High flow nasal cannula.

Table 2 Matrix of Phi coefficient for binary outcomes

	Death	ICU	BiPAP	VENT	HFNC
Death	1	-	-	-	-
ICU	0.67	1	-	-	-
BiPAP	0.71	0.70	1	-	-
VENT	0.60	0.94	0.65	1	-
HFNC	0.58	0.48	0.67	0.43	1

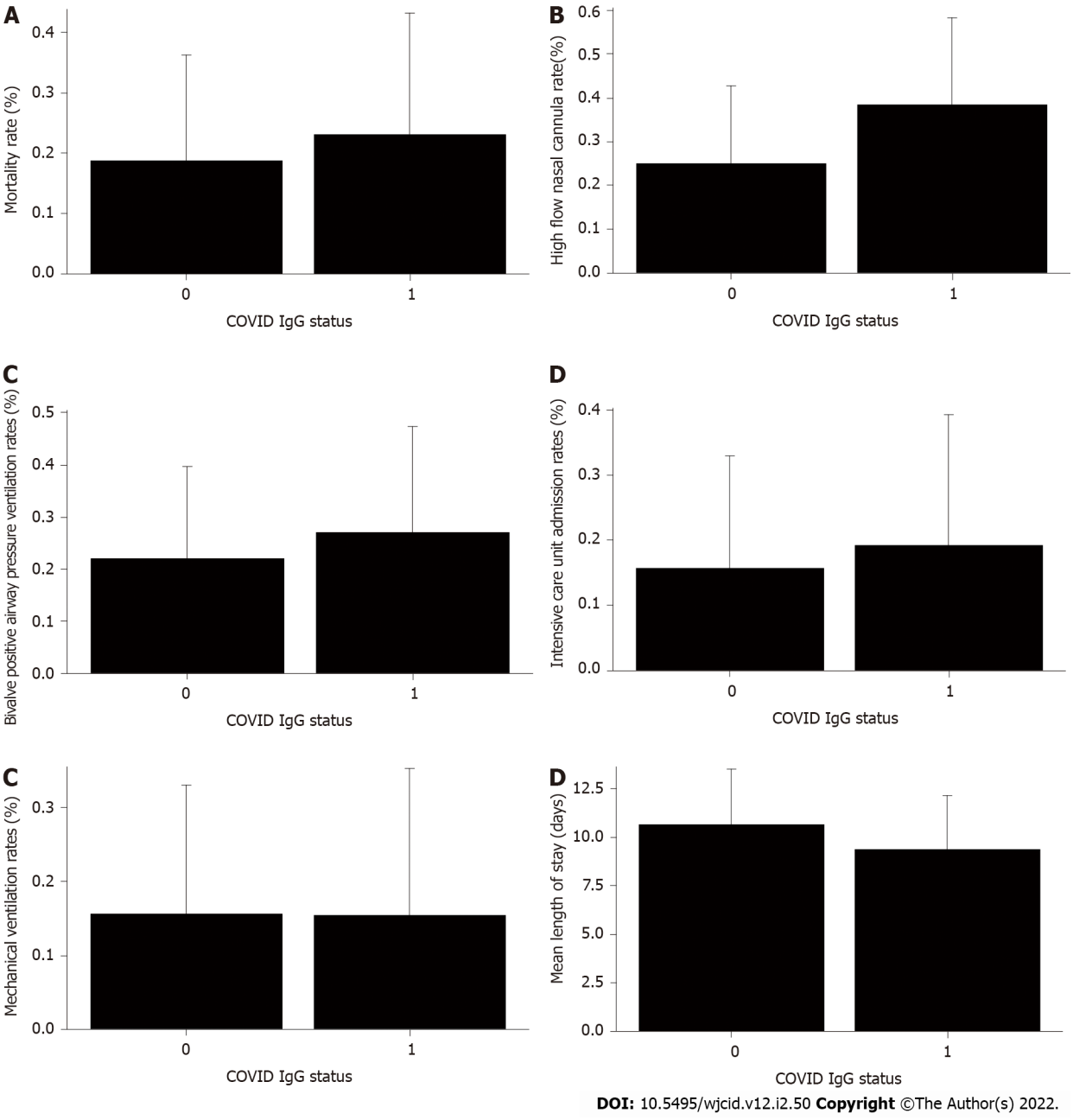
ICU: Intensive care unit; BiPAP: Bilevel positive airway pressure ventilation; VENT: Mechanical ventilation; HFNC: High flow nasal cannula.

Outcomes by other factors

For death, only age (numerical) was a significant predictor (F value = 5.07, P value = 0.0283). As age increased, the probability of having an endpoint of one (Death) increased (Figure 2). No other variables for any of the outcomes were significant.

DISCUSSION

SARS-CoV-2 infection causes an atypical pneumonia like respiratory illness known as COVID-19 characterized by fever, dyspnea, anosmia and a worsening hypoxia[21,22]. Among those hospitalized with COVID-19, patients often required supplemental oxygen using HFNC, BiPAP and an increased admission into the ICU requiring mechanical ventilation depending on the severity of the respiratory failure and lung parenchymal involvement. The pathophysiology of COVID-19 is primarily an immune-mediated process with a variety of antibody signatures among which the IgG signatures were of interest to our study. A robust immune-mediated inflammatory cascade guides the pathophysiology of the COVID-19 illness[22-24]. Different proinflammatory cytokines such as IL-6 and TNF-α have been



DOI: 10.5495/wjcid.v12.i2.50

Figure 1 Clinical outcomes across Covid IgG status (0 = negative, 1 = positive). A: Mortality rates; B: High flow nasal cannula rates; C: Bilevel positive airway pressure ventilation rates; D: Intensive care unit admission rates; E: Mechanical ventilation rates; F: Mean length of stay. COVID: Coronavirus disease.

correlated with diseases severity[25].

There is some data available to understand the humoral response to SARS-CoV-2 infection and role of various IgG subtypes in the body's line of defense. Much of it was inherited from the studies of SARS-CoV-1[26]. IgG antibodies directed towards the spike protein (IgG-S) and that of the nucleocapsid protein (IgG-N) are the two important components of humoral immunity against SARS-CoV-2 infection. SARS-CoV-2 uses the spike protein to bind to the target cell through its receptor-binding domain and therefore is the target site for neutralizing antibody, IgG-S[27]. The role of IgG-S in early viral clearance is crucial for favorable clinical course and survival[28-30]. IgG-S is considered the neutralizing antibody which may elicit a protection against SARS-CoV-2 by interfering with virion binding to host cell receptors, blocking cellular uptake and preventing endosomal processing of viral genome[13,27]. However, the kinetics of the antibody response becomes more complex to understand with current available literature, which is conflicting. In one interesting study, there is a link between the IgG-S response and COVID-19 severity, but the antibody response has to develop in a specific time window to improve viral clearance and disease outcomes. A faster antibody response was associated with better survival (within the first 14 d of infection) and deceased patients showed a slower antibody response although they reached higher IgG titers later in the disease trajectory[31]. Other studies have shown that, severely ill patients exhibit higher peak, faster and stronger antibody response compared to mildly

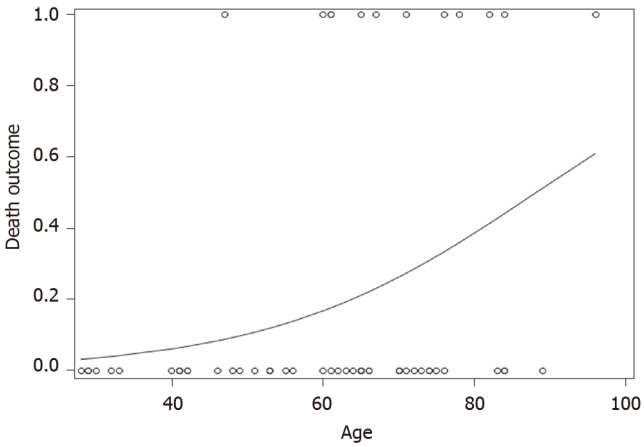


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Figure 2 Logistic regression results of death outcome by age (Death = 1, Recovery = 0) by age (numerical). As age increased, the probability of mortality increased (*F* value = 5.07, *P* value = 0.0283).

symptomatic patients[13,32]. Severely ill COVID-19 patients have been found to produce a unique serologic signature with increased IgG-S with afucosylated Fc glycans. The Fc modification of IgG-S triggers activation of natural killers cells and enhances production of IL-6 and TNF-α by primary monocytes that results in more severe disease[33].

The role of IgG-N in the pathogenesis and clinical course of COVID-19 remains largely unknown. As to our current knowledge, severe COVID-19 is characterized by a series of inflammatory signatures including a cytokine storm, inflammatory alveolar infiltrates and formation of vascular microthrombi [33]. During the peak of the pandemic, clinicians took their chance to use different inflammatory markers such as C-reactive protein, platelet count, D-dimer and Ferritin, to name a few to monitor diseases progression and crisis planning. However, data to support the specificity of these inflammatory signatures as reliable prognostic markers for COVID-19 is limited[34,35]. As per one report by Batra *et al* [19], showed that titers of IgG-N at the time of admission can be a prognostic factor in the clinical course of the diseases and was associated with increased incidence of hypoxemia, admission into the ICU and extended length of stay in the hospital. In our study, we hypothesized that the presence of IgG-N at the time of admission into the hospital could be used as a marker of impending diseases severity and determine hospital course. We pursued a qualitative measurement of IgG-N on all our patients. Some key parameters such as the degree of hypoxemia, mean length of hospitalization, ICU admission, need for mechanical ventilation and patient outcome as in-hospital datasets were examined in our study group. We enrolled a total of 59 patients who were admitted with hypoxia secondary to COVID-19, out of which 26 (44%) patients had IgG-N antibody at the time of admission into the hospital. Our goal was to investigate the role of IgG-N as a marker to anticipate the clinical course in hospitalized patients. Based on our results, we concluded that IgG-N might not be a reliable predictor of COVID-19 diseases severity.

Our data indicate that age was a single independent predictor of death following hospitalization, which is in support of reports published earlier[36,37]. As age increased, the probability of death increased (Figure 2). Mortality rate was not significantly different in IgG-N positive group *vs* negative (Figure 1A). We did not find any statistical difference with the need to use HFNC between the two groups (Figure 1B). Many of our patient population had clinically progressive diseases with worsening respiratory failure requiring BiPAP or transfer to ICU to be intubated and placed on mechanical ventilation. After following the patient pool until discharge, we did not find any significant difference with the need to use BiPAP between the two groups with and without IgG-N at the time of admission (Figure 1C). The admission rate into the ICU and need for mechanical ventilation was not statistically significant either (Figures 1D and E). Although we saw an extended LOS among the IgG-N negative group, but after adjusting for the extreme outlier, the findings were no longer significant (Figure 1F). Furthermore, median LOS was actually lower in the IgG-N negative group, showing that the extreme value was skewing the LOS average.

Our study had several limitations. We did not measure the IgG-N antibody titers in our study and so we cannot imply if high *vs* low antibody titers have any direct impact on the disease severity and mortality in COVID-19. Since every individual patient was enrolled into the study only when they were symptomatic enough to meet criteria for hospitalization, especially hypoxic with oxygen saturation < 90%, it could be argued that they may be at different stage of the diseases course and different phase of the seroconversion. This could have confounded our findings since seroconversion and viral kinetics are time dependent phenomena. We did not standardize our patients based on their underlying comorbidities, which further could have influenced our results. More investigation using a larger

sample size and different IgM/IgG subtypes is warranted to put more light in this area.

CONCLUSION

We have analyzed the presence or absence of IgG-N in patients admitted to the hospital with severe or critical COVID-19 illness and evaluated the effects of presence of IgG-N on clinical severity and outcome. Age happens to be the single independent risk factor for a worse outcome. Our analysis revealed no significant correlation between IgG-N status and degree of respiratory failure or mortality. The degree of respiratory failure was characterized by the utilization of high flow nasal canula, bilevel positive pressure ventilation and intubation with mechanical ventilation. IgG-N seroconversion had no significant effect on mean length of stay in the hospital. Further studies with large cohorts and risk-adjusted comorbidities are needed to demonstrate the more accurate role of IgG-N seroconversion on clinical outcome.

ARTICLE HIGHLIGHTS

Research background

During the peak of the coronavirus disease 2019 (COVID-19) pandemic, hospitals and clinicians had to adapt quickly to the rapid spread of the infection in the community. In the absence of adequate literature, clinicians hypothesized and studied the utility of various protein markers to prognosticate their patients. We intended to study the correlation of anti-nucleocapsid-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibody (IgG-N) presence with the clinical outcome in severely ill hospitalized COVID-19 patients.

Research motivation

We were interested in characterizing a correlation between presence or absence of IgG-N and clinical outcome in hospitalized COVID-19 patients. We wanted to test the ability of IgG-N in predicting the severity of illness, maximal oxygen support needed and final outcome in order to mobilize staff, manage intensive care unit (ICU) beds and ventilators as a part of the crisis management planning.

Research objectives

To study the effect of SARS-CoV-2 anti-nucleocapsid-IgG on COVID-19 diseases severity and outcome. We studied the effect of presence or absence of IgG-N on mean length of stay in the hospital, maximal oxygen support needed and mortality.

Research methods

We conducted a retrospective cohort study on adults aged between 28-96, being admitted to the hospital with severe or critical COVID-19 illness. Blood sample was collected either at or within 48 h of admission to the hospital to check for SARS-CoV-2-IgG-N. A total of fifty nine patients were enrolled into the study. We utilized a binary logistic regression model to analyze the outcome data.

Research results

Our data demonstrated that the need for maximal oxygen support, mean length of stay in the hospital and mortality were not significantly different between the groups with or without IgG-N at the time of admission.

Research conclusions

We concluded that IgG-N seroconversion had no significant correlation with the need for maximal oxygen support as well as mortality during the course of hospitalization. Length of stay in the hospital was not significantly different across the IgG-N status.

Research perspectives

Our study demonstrated that presence of anti-nucleocapsid-IgG against SARS-CoV-2 infection had no impact on the clinical outcome or diseases severity in hospitalized COVID-19 patients. We did not find a correlation of statistical significance to use IgG-N as a biomarker to predict clinical outcome in COVID-19 patients admitted to a community hospital in North Dakota. Acknowledging the limitation of our study, we look forward to a future study with larger sample size and risk-adjusted comorbidities to investigate the association with better clarity.

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FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Five-year retrospective hospital-based study on epidemiological data regarding human leishmaniasis in West Kordofan state, Sudan

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Abstract

BACKGROUND

Leishmaniasis is a neglected zoonotic disease, endemic in Sudan. Estimating this disease is very important to inform the health care policymakers and the governments to apply proper health and economic policies.

AIM

To find out the frequency and distribution of human leishmaniasis based on sex and age for 5 years in the West Kordofan state, Sudan.

METHODS

A 5-year retrospective study from 2016 through 2020 was carried out using local hospital records of leishmaniasis patients. The positive results were recorded after

performing at least one of the following leishmaniasis standard tests: direct agglutination test, enzyme-linked immunosorbent assay and leishmania skin test. The sex and age of each patient were recorded. The collected data were analyzed using STATA package version 16.

RESULTS

A total of 162443 patient records from 2016 to 2020 were retrieved. Of these, 4.39% were found to be positive for leishmaniasis. The disease has been more common in males (65.3%) than in females (34.7%). The highest reported prevalence (6.58%) was in patients 15-44 years, and the lowest prevalence (1.95%) was among patients ≥ 65 years.

CONCLUSION

The results of the current study indicate that leishmaniasis is endemic in the study area even though the numbers of patients in the 5 consecutive years were varying. In addition, the disease was common in males and adults. The interpretation of these findings should take into consideration the absence of information about some important confounding factors.

Key Words: Epidemiology; Human Leishmaniasis; West Kordofan; Sudan; Endemicity

Core Tip: A 5-year retrospective study was conducted to find the frequency and distribution of human leishmaniasis in the West Kordofan state and was based on sex and age. A total of 162443 patient records were retrieved. Of these, 4.39% were found to be positive for leishmaniasis. The disease has been more common in males than in females. The highest reported prevalence was in patients 15-44 years, and the lowest prevalence was among patients ≥ 65 years. The current study indicates that leishmaniasis is endemic in the study area even though the numbers of patients in the 5 consecutive years were varying.

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INTRODUCTION

Leishmaniasis is a parasitic zoonotic disease caused by the *Leishmania* parasite[1]. The disease is mainly transmitted by the bite of infected female phlebotomine sandflies[2]. The World Health Organization classified the disease as a neglected tropical disease[2,3]. There are several forms of human leishmaniasis, and the most common forms are cutaneous leishmaniasis (CL), which causes skin sores, and visceral leishmaniasis (VL), which affects several internal organs (usually the spleen, liver and bone marrow)[4]. All forms of the disease have been strongly associated with poor socioeconomic status, population displacement, a weak immune system and climate change[5-8]. Leishmaniasis cases have been reported in almost all continents in about 89 countries, with an estimated 700000 to 1 million new cases occurring annually. Most cases occur in East Africa, Southeast Asia and South America[4,9]. Outbreaks of human leishmaniasis worldwide were reported from East African countries namely Sudan, South Sudan and Ethiopia[10-15].

Sudan is a highly endemic country for leishmaniasis (both CL and VL). The disease represents a serious health problem that may affect the whole healthcare system[16]. The geographical distribution of the disease in Sudan have a high relation to the distribution of the vectors. Studies revealed that VL is endemic in the savannah area, which starts from the Gadarif state in the east to the White Nile State in the west and from the Kassala state in the northeast to the Blue Nile State in the south. Also, VL was reported in some scattered foci in the Kordofan state and Darfur state. Moreover, CL is found in a fluctuating pattern mainly in the northern, central and western parts of the country[17-26].

West Kordofan is the 18th state of Sudan. It was established in July 2013 on the border with the Republic of South Sudan in the east, North Kordofan state in the North and South Darfur state in the west. People of West Kordofan, especially the Mesairya tribe, continuously move to and from South Sudan where leishmaniasis disease is endemic[7]. The state also contains many south Sudanese refugee camps spread almost all over the state. The geographical location together with the high presence of the suspected infected refugees makes the people of the West Kordofan state very vulnerable to leishmaniasis (for both CL and VL). A community-based study in two West Kordofan cities, namely

Muglad and Babnousa, reported that out of 1781 randomly selected volunteers, 238 persons (13%) tested positive for leishmaniasis[27]. Based on that, there is still a need for a deeper look at the epidemiology of the disease in the whole state, in both males and females and in all age groups, to design and implement suitable prevention and eradication programs for the disease at the state level. Thus, this study aimed to find out the frequency and distribution of human leishmaniasis based on sex and age in the West Kordofan state for 5 years.

MATERIALS AND METHODS

The present retrospective study was conducted among patients who were admitted to any hospital in the West Kordofan state, Sudan from January 1, 2016 to December 31, 2020 to test the presence of human leishmaniasis of any type in the population of West Kordofan. In addition to the clinical symptom and signs, the positive results were recorded after performing at least one of the following leishmaniasis standard tests: direct agglutination test, enzyme-linked immunosorbent assay and leishmania skin test. Data of age, sex and presence of any type of leishmaniasis were retrieved from the medical records department in the Ministry of Health West Kordofan, with the approval of the ministry ethical committee. The medical record department follows the guidelines of the International Classification of Diseases 10 coding.

Statistical analysis

Descriptive statistics and data analysis were done using STATA package version 16 (Stata Corp LLC, College Station, TX). *Z* test was applied to compare the proportions between the study groups. If the *P* value was less than or equal to 0.05, it indicated that there was a significant difference between the proportions of the two groups.

RESULTS

A total of 162443 patient records (87847 female and 74596 male patients) from 2016 to 2020 were retrieved. Of these, 4.39% were found to be positive for leishmaniasis. Among them, 34.7% were females and 65.3% were males. The diagnostic prevalence of the infection was first found to be very low in 2016 (2.57%). After 1 year in 2017, the highest reported prevalence of 5.83% was observed and then started to decrease (with some fluctuation) to 3.67% in 2020 (Figure 1).

Sex-related differences in leishmaniasis prevalence are presented in Table 1. The prevalence was significantly higher ($P \leq 0.05$) in males compared to females in the period from 2017 to 2020, while in 2016 there was no significant variation between the sexes ($P > 0.05$). The prevalence of leishmaniasis was relatively increased with participant age in both females and males. The prevalence reached its peak in patients 15-44 years, which was 6.58%, then decreased to be the lowest of 1.95% among patients ≥ 65 years (Tables 2 and 3). In addition to that in all age groups, males had a higher prevalence of leishmaniasis than females.

DISCUSSION

Leishmaniasis is an endemic neglected zoonotic disease in Sudan, widespread all over the country from the eastern states to the western states and from southern states to northern states[16]. However, few data about the epidemiological and demographical distribution of the disease in western states is available, especially in West Kordofan, and it seems to be overlooked[20,24,25,27]. Thus the current study is the first comprehensive attempt to describe the epidemiological and demographical distribution of the disease in the state.

In this study, the data on human leishmaniasis was collected from the annual health statistical reports for 5 years (2016–2020) and was analyzed to show the burden of the disease in the West Kordofan state, Sudan. The results highlight that a total of 162443 people were admitted to the hospitals and health care centers in the state. Of these, 7128 people were infected during this period. In 2016 the prevalence of leishmaniasis was found to be very low at 2.57%. Surprisingly, it was raised to 5.83% in 2017, and from then it seemed to decrease. The reason could be that the government of Sudan in collaboration with the World Health Organization and other related international organizations developed diagnostic and control strategies to limit the spread of the disease in October 2014[28,29]. The first 2 years (2015 and 2016) were for training the health care professionals in the state on the new diagnostic and prevention methods. That may explain the low prevalence in the 1st study year because of the use of the low sensitivity diagnostic test. Then after implementing the new diagnostic method in 2017 the rate was raised. In line with that, after 2017 the prevalence of leishmaniasis was decreasing because of implementing the new control strategies.

Table 1 Sex distribution of different patients infected with leishmaniasis classified by year

Year	Female, *n* (%)	Male, *n* (%)	Total, *n* (%)	*P* value
2016	244 (1.85)	405 (3.37)	649 (2.57)	0.2277
2017	780 (3.99)	1322 (7.99)	2102 (5.83)	0.0002
2018	621 (3.45)	1142 (7.49)	1763 (5.30)	0.0002
2019	409 (2.36)	941 (5.83)	1350 (4.03)	0.0015
2020	420 (2.12)	844 (5.77)	1264 (3.67)	0.0008
Total	2474 (2.82)	4654 (6.24)	7128 (4.39)	0.0001

Table 2 Age and sex distribution of patients infected with leishmaniasis

Age group	Female, %	Male, %	Total, %	*P* value
< 1 yr	2.22	5.05	3.52	0.0001
1-4 yr	3.93	4.5	4.19	0.2523
5-14 yr	5.47	7.89	6.57	0.0001
15-44 yr	5.63	7.68	6.58	0.0001
45-64 yr	2.81	4.42	3.55	0.0012
≥ 65 yr	1.73	2.2	1.95	0.3452

Table 3 Comparing the sex-wise proportion of human leishmaniosis reported in each age group

Year	Sex	Age group					
	Females	< 1%	1%-4%	5%-14%	15%-44%	45%-64%	≥ 65%
2016		1.19	2.66	4.52	3.51	1.85	1.06
2017		2.16	1.80	6.00	4.97	2.47	2.01
2018		1.44	3.35	4.97	5.55	2.39	1.55
2019		3.54	3.90	5.54	6.81	2.98	1.77
2020		2.51	4.83	5.98	6.73	4.04	2.03
Total		2.22	3.93	5.47	5.63	2.81	1.73
2016	Males	0.92	3.81	6.59	5.20	2.30	1.19
2017		2.06	5.26	8.30	6.90	3.70	2.35
2018		3.26	3.69	7.64	8.50	3.81	2.08
2019		4.64	3.75	6.90	7.20	5.08	2.12
2020		14.11	5.90	9.84	10.23	6.84	3.14
Total		5.05	4.50	7.89	7.68	4.42	2.20

The current study found that the overall prevalence of leishmaniasis in West Kordofan was lower than that reported by Sharief *et al*[27] in 2019. This may be due to the difference in sample size and study period, which were bigger and longer, respectively, in the current study compared with the other study. Nevertheless, the study area could have a great impact on the result. In their study, Sharief *et al* [27] collected data in two districts in the state, but the current study collected data from all 14 districts.

Sex-related distribution of human leishmaniasis in the study revealed that males were highly affected compared to females with an overall percentage of 65.3% and 34.7%, respectively. This is in line with Awadalla *et al*[30], Ebrahim *et al*[25] and Collis *et al*[31] and disagrees with Mohammed *et al*[20]. This result might be justified because the majority of males are nomads. They are moving seasonally to the tropic and subtropic areas in South Sudan whereby the exposure to the risk of sandflies bites is high. The same exposure of males in different agricultural areas may be a contributing factor to the infections. Consequently, males are more vulnerable than females.

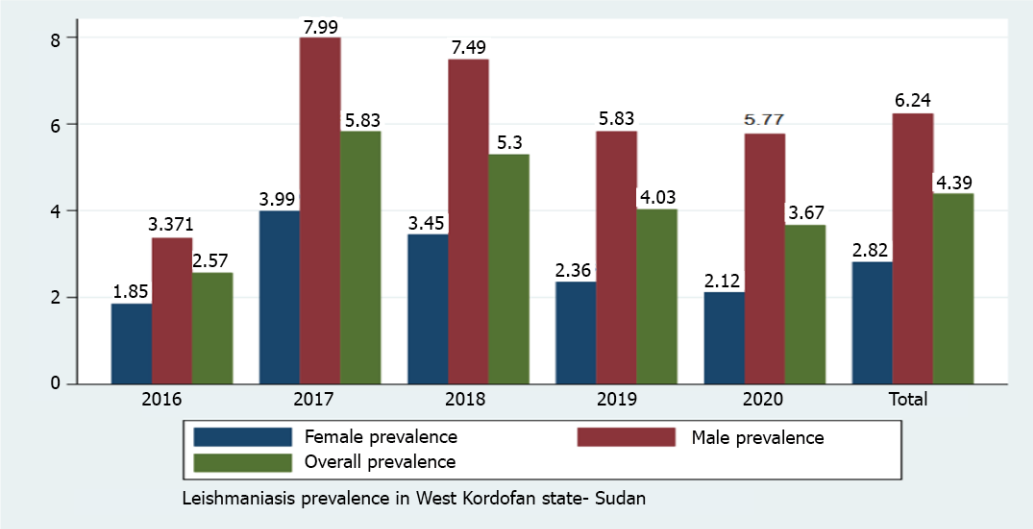

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Figure 1 Prevalence of leishmaniasis during 2016-2020 according to sex.

Age-wise distribution found that people in the age group 15-44 had the highest prevalence among all populations. Similar results were reported by Awadalla *et al*[30], Osman *et al*[24], Ebrahim *et al*[25] and Collis *et al*[31]. These studies indicated that the adult men and women aged between 15-44 years were more affected by the disease compared to the lower and higher age groups. This can be put in the context that this age group is the working-age group in all fields, especially the agricultural field. In contrast, a study conducted by Mohammed *et al*[20] indicated that the most affected age groups were children between 1-year-old and 5-years-old.

In addition, the lowest reported prevalence in this study was found in the age group > 65 years. Although this group of people is more vulnerable to infections because the immune system weakens, they have a relevant low prevalence of the disease. The possible reason that these patients might have less exposure to the infection is due to their lifestyle, which keeps them away from the areas where the carrier host exists, especially in the agricultural areas.

This study provided important epidemiological information about human leishmaniasis in West Kordofan, which is missing from the scientific literature despite its urgent need to design a collaborative effort and immediate action by policymakers and governments (federal and state government) for prevention and eradication programs in light of the one health concept. However, the absence of data about the infection (type, site and status), *Leishmania* parasite and other potential risk factors in some included studies are considered as limitations of the current study.

CONCLUSION

The results of the current study indicate that leishmaniasis is endemic in the study area even though the numbers of patients in the 5 consecutive years were varying. In addition, the disease was common in males and adults. The interpretation of these findings should take into consideration the absence of information about some important confounding factors. Further studies need to be carried out to clarify the economic impact of the disease on the public health sector in the state and the role of domestic animals in the epidemiology of the disease in Sudan.

ARTICLE HIGHLIGHTS

Research background

In Sudan, human leishmaniasis is endemic, and the prevalence of the disease varies throughout the country. Although the disease in Sudan is serious, there is no overall estimation of the prevalence of human leishmaniasis in the western parts of the country, especially in the West Kordofan state.

Research motivation

The lack of published studies about human leishmaniasis in the western parts of Sudan especially in the West Kordofan state may cause a problem for the policymakers and local governments to develop and

adopt a suitable prevention program to deal with the disease at the state level and the country level.

Research objectives

The objective of this study was to find the frequency and distribution of human leishmaniasis based on sex and age in West Kordofan, Sudan for 5 years.

Research methods

A 5-year retrospective study from 2016 through 2020 was carried out using local hospital records of leishmaniasis patients. The positive results were recorded after performing at least one of the following leishmaniasis standard tests: direct agglutination test, enzyme-linked immunosorbent assay and leishmania skin test.

Research results

A total of 162443 patient records from 2016 to 2020 were retrieved. Of these, 4.39% were found to be positive for leishmaniasis. The disease has been more common in males (65.3%) than in females (34.7%). The highest reported prevalence (6.58%) was in patients 15-44 years, and the lowest prevalence (1.95%) was among patients ≥ 65 years.

Research conclusions

The results of the current study indicate that leishmaniasis is endemic in the study area even though the numbers of patients in the 5 consecutive years were varying. In addition, the disease was common in males and adults.

Research perspectives

Further studies need to be carried out to clarify the economic impact of the disease on the public health sector in the state and the role of domestic animals in the epidemiology of the disease in Sudan.

FOOTNOTES

Author contributions: Abdulslam Abdullah A, Ahmed M, Gadeed A, Eltayeb A, Ahmed S, Hamad S and Hussein M conceived and designed the study and directed implementation and data collection; Abdulslam Abdullah A, Ahmed M and Hamad S analyzed and interpreted the data and drafted the manuscript; Abdulslam Abdullah A, Ahmed M, Gadeed A, Eltayeb A, Ahmed S, Hamad S and Hussein M edited the manuscript for intellectual content and provided critical comments on the manuscript; All authors gave final approval of the version to be published, have agreed on the journal to which the article has been submitted and agreed to be accountable for all aspects of the work.

Institutional review board statement: Ethical approval and permission were obtained from the Ministry of Health West Kordofan Ethics Review Committee.

Informed consent statement: Individual consent was not required as the data used were secondary, collected from the Ministry of Health West Kordofan data center.

Conflict-of-interest statement: All authors report no relevant conflict of interest for this article.

Data sharing statement: The data that support the findings of this study are available at the Ministry of Health West Kordofan but restrictions apply to the availability of these data, which were used under license for the current study and are not publicly available. Data are however available from the authors upon reasonable request and with the permission of the Ministry of Health West Kordofan.

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CASE REPORT

Incidental diagnosis of intestinal spirochetosis in a patient with chronic hepatitis B: A case report

Samantha Novotny, Joseph Mizrahi, Eric U Yee, Michael J Clores

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Abstract

BACKGROUND

Intestinal spirochetosis (IS) is caused by *Brachyspira* colonization of the gastrointestinal tract. Some patients are asymptomatic, while others present with gastrointestinal complaints such as abdominal pain, diarrhea, or gastrointestinal bleeding. However, the clinical significance of asymptomatic IS is unclear, and guidelines are lacking regarding decision to treat.

CASE SUMMARY

A 73-year-old male with peptic ulcer disease and gastroesophageal reflux was evaluated for elevated liver enzymes. He was diagnosed with chronic hepatitis B virus and prescribed entecavir. Additionally, he was leukopenic and had stage 4 liver fibrosis on transient elastography. After 5 mo, the patient returned for esophagogastroduodenoscopy and screening colonoscopy. He denied any gastrointestinal symptoms at that time. Findings included grade I distal esophageal varices, mild portal hypertensive gastropathy, and patchy nodular gastric antral mucosa. On colonoscopy, several polyps were removed. Hematoxylin and eosin stain of mucosa adjacent to the polyps revealed a "false brush border," and Steiner stain identified spirochetes adherent to the mucosa. These pathology findings confirmed the diagnosis of IS. He was managed conservatively with careful observation and without antibiotic therapy *via* a multidisciplinary approach between gastroenterology and infectious disease. He remained asymptomatic at the 7-wk follow-up.

CONCLUSION

This case reports the finding of incidental, asymptomatic IS in a leukopenic

patient with hepatitis B virus. Conservative management was appropriate.

Key Words: Intestinal spirochetosis; Hepatitis B; Colonoscopy; Histology; Leukopenia; Case report

Core Tip: Intestinal spirochetosis is caused by *Brachyspira* colonization of the gastrointestinal tract. Some patients are asymptomatic, while others present with gastrointestinal complaints such as abdominal pain, diarrhea, or gastrointestinal bleeding. However, the clinical significance of asymptomatic intestinal spirochetosis is unclear, and guidelines are lacking regarding decision to treat. We report the case of an asymptomatic 73-year-old male with chronic hepatitis B and leukopenia who was incidentally diagnosed with intestinal spirochetosis on pathology of polyps resected during routine screening colonoscopy. He was managed conservatively with careful observation and without antibiotic therapy *via* a multidisciplinary approach between gastroenterology and infectious disease.

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INTRODUCTION

Intestinal spirochetosis (IS) is a condition of intestinal colonization with *Brachyspira* species, typically *Brachyspira aalborgi* or *Brachyspira pilosicoli*[1]. The prevalence of IS varies with geographic location. Estimates suggest a prevalence of 11%-64% in developing countries and 1%-5% in North America and Europe[2]. Colonization is more common in people with HIV and men who have sex with men[1]. Patients with IS are more often male, and the mean age at diagnosis is 51[3]. The mode of transmission is not clear; however colonization may result from exposure to infected water, animals, birds, or feces [4]. A literature review was performed focusing on asymptomatic or incidental IS with the following search terms: Intestinal spirochetosis, intestinal spirochaetosis, colonic spirochetosis, colonic spirochaetosis, asymptomatic, and incidental. Only reports describing adults without gastrointestinal symptoms and including decision to treat were considered. This search revealed a paucity of literature describing the clinical implications of infection or evidence-based treatment recommendations in asymptomatic patients with IS. We present the case of an asymptomatic patient with an incidental finding of IS during colonoscopy and discuss the management strategy.

CASE PRESENTATION

Chief complaints

A 73-year-old male presented to our gastroenterology practice for follow-up esophagogastroduodenoscopy (EGD) 5 mo after an initial EGD. He simultaneously underwent screening colonoscopy as his last colonoscopy was more than 10 years prior. He was feeling well and reported no gastrointestinal symptoms.

History of present illness

This patient initially underwent EGD 5 mo prior to this visit due to melena and symptomatic anemia. EGD findings were notable for Los Angeles Grade A esophagitis and a large, cratered gastric antral ulcer with pigmented spots. He was diagnosed with peptic ulcer disease and gastroesophageal reflux and was discharged on pantoprazole 40 mg twice daily. At that time, a workup for abnormal liver enzymes revealed a new diagnosis of chronic hepatitis B virus (HBV). He tested negative for HIV, and entecavir was eventually initiated. Transient elastography showed stage 4 liver fibrosis. He was also leukopenic with a white blood cell count ranging from 2700 to 3900.

History of past illness

Additional medical history was notable for hypertension. He denied previously undergoing diagnostic workup for congenital immunodeficiencies. Medications included vitamin D_3 50000 units oral daily and folic acid 1 mg oral daily.

Personal and family history

Pertinent social history included a history of military service with international travel to Guantanamo Bay, Cuba, and Greece, and remote alcohol and tobacco use. He had one tattoo that was obtained 50 years prior. He denied recent or remote history of unprotected sexual intercourse and denied history of sexually transmitted diseases. Family history was non-contributory.

Physical examination

The patient was evaluated 1 wk prior to his EGD and colonoscopy, at which time he was afebrile and mildly hypertensive to 148/80. Body mass index was 28.6. The patient's exam was benign, with a soft, non-tender, and non-distended abdomen. Bowel sounds were present and normal.

Laboratory examinations

Laboratory results are shown in Table 1. Notably, ALT was 72 and AST was 57, recorded 5 mo prior to this visit. Prothrombin time and activated partial thromboplastin time were within normal limits at that time. ALT and AST decreased to 41 and 39, respectively, 1 wk prior to this visit. Alkaline phosphatase, bilirubin, total protein, and albumin levels remained within normal limits. He was leukopenic, thrombocytopenic, and had a normocytic anemia 1 wk prior. Infectious disease workup 5 mo prior revealed a positive HBV DNA, positive HBV surface antigen, and negative HBV E Antigen. The patient was retested 1 wk prior to this visit, revealing positive HBV DNA, positive HBV total core antibody, positive HBV E antibody, and negative HBV core IgM.

Imaging examinations

EGD revealed grade I varices in the distal esophagus, irregular Z-line, mild portal hypertensive gastropathy, and patchy nodular mucosa in the gastric antrum. No ulcers were seen. Colonoscopy revealed multiple small polyps that were resected, diffuse diverticulosis, and non-bleeding hemorrhoids (Figure 1).

MICROBIOLOGICAL IDENTIFICATION

Pathology results of the resected colon polyps showed tubular adenomas, a sessile serrated lesion, and a hyperplastic polyp. Incidentally, a hematoxylin and eosin stain of the colonic mucosa adjacent to the polyps identified intestinal spirochetosis appearing as a "false brush border" (Figure 2A), with a Steiner stain confirming the presence of spirochetes (Figure 2B).

FINAL DIAGNOSIS

The final diagnosis in this case is asymptomatic IS.

TREATMENT

In this case of asymptomatic IS, the patient was managed conservatively without antibiotics.

OUTCOME AND FOLLOW-UP

The patient followed up with both gastroenterology and infectious disease specialists. He remained asymptomatic at the 7-wk follow-up, and a repeat HIV screen at that time was negative. Thus, he was not prescribed antibiotics and was closely followed for development of any gastrointestinal symptoms.

DISCUSSION

We describe an asymptomatic case of IS diagnosed *via* histology of tissue obtained during routine colonoscopy. Histologic findings in IS classically include a "brush-like" appearance of organisms oriented perpendicular to the epithelial surface of the intestine[5]. This is consistent with the findings seen on stains of our patient's colonic tissue. A large study found that 90% of IS biopsies showed no changes on histology other than the presence of spirochetes[6]. However, there have been several reports of histologic changes, notably inflammation with macrophages, neutrophils, eosinophils, and lymphoid follicles on biopsy[6-8]. Our patient represents a case of isolated IS, with identification of

Table 1 Laboratory results at 5 mo and 1 wk prior to colonoscopy

Parameter	5 mo prior	1 wk prior
ALT	72	41
AST	57	39
Alkaline phosphatase	77	88
Total bilirubin	0.9	0.5
Direct bilirubin	Not obtained	0.2
PT/INR	12.9/1.2	Not obtained
aPTT	29.1	Not obtained
Total protein	7.2	7.0
Albumin	3.7	3.8
WBC count	3.9	3.1
Hemoglobin	9.4	10.2
Hematocrit	27.3	30.6
Mean corpuscular volume	96	85
Platelet count	111	104
HBV DNA quantitative viral load	8.22 log IU/mL	8.14 log IU/mL
HBV surface antigen	Positive	Not obtained
HBV core total antibody	Not obtained	Positive
HBV core IgM antibody	Not obtained	Negative
HBV E antigen	Negative	Not obtained
HBV E antibody	Not obtained	Positive

ALT: Alanine transaminase; AST: Aspartate transaminase; PT: Prothrombin time; INR: International normalized ratio; aPTT: Activated partial thromboplastin time; WBC: White blood cell; HBV: Hepatitis B virus.

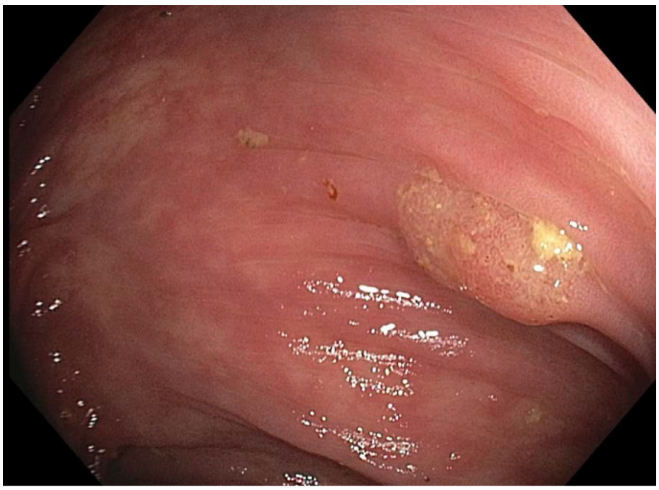

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Figure 1 Colonoscopy image of polyp in the transverse colon.

spirochetes without any changes on the cellular level.

Additionally, this report describes a case of IS associated with a hyperplastic polyp, tubular adenomas, and a sessile serrated lesion. On colonoscopy, some patients with IS have no remarkable findings, while others have had polyps, mucosal erosions, or ulcerations[5,9]. Several case reports describe findings of IS in patients with colon polyps of varying histology (including adenomatous,

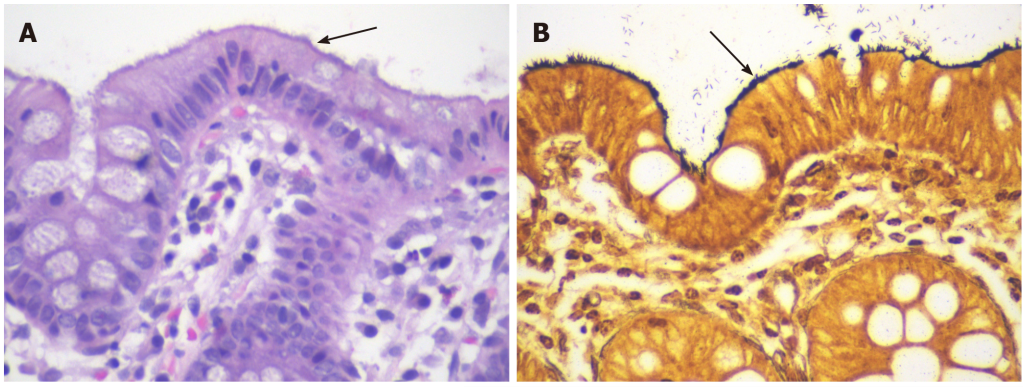

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Figure 2 Pathology images of samples taken during colonoscopy. A: Hematoxylin and eosin stain (× 600 magnification) showed intestinal spirochetosis appearing as a basophilic, fuzzy lining over the luminal surface of colonocytes ("false brush border"); B: Steiner stain (× 600 magnification) highlighted spirochetes overlying the luminal surface.

hyperplastic, sessile serrated lesions, and other polyps)[10-14].

IS is often an incidental finding, and its clinical implications are unclear. Several reports describe asymptomatic patients found to have spirochete colonization[11,15-18]. However, other reports have described the presence of various gastrointestinal symptoms including diarrhea, changes in bowel habits, abdominal pain, and overt or occult gastrointestinal bleeding[12,17,19-21]. A study of 209 patients with IS found 46% of patients reported abdominal pain, 51% diarrhea, and 13% alternating constipation and diarrhea[3].

Notably, IS has been frequently reported in immunocompromised patients, such as those with HIV or taking immunosuppressive drugs[22-24]. There are additional case reports of IS in patients with chronic HBV[5] and hepatitis C[8,25]. In the present case, leukopenia in the setting of chronic liver disease secondary to HBV may have played a role in the development of spirochete colonization. The origin of this patient's HBV infection is not certain. His history is notable for having one tattoo, but he denied sexual or military exposures that would otherwise suggest a source for his HBV infection.

As the clinical significance of IS is controversial, need for treatment has been debated. Recommendations from the 2021 European Academy of Dermatology and Venereology Guidelines support treatment for IS with metronidazole 500 mg twice daily or 250 mg three times daily for a 14-d course[1]. However, this recommendation does not differentiate between symptomatic and asymptomatic patients. A large study found that 40% of IS patients received treatment, and of these 86% were treated with metronidazole. However, only 52% of treated patients reported improvements in symptoms[3]. In an earlier study, 17 patients were treated with metronidazole 500 mg three times daily for 10 d, and 15 patients had resolution of symptoms[9]. Evidence is lacking for treatment guidelines in the asymptomatic population. A comprehensive literature search identified a limited number of publications reporting decision to treat in 5 cases of asymptomatic adults with IS. Of these cases, 4 patients were not treated[5,11,16,18]. The fifth patient was treated with metronidazole and experienced resolution of the IS infection[15]. Due to our patient's continued lack of symptoms, he was not treated with antibiotics and is being managed with close follow-up.

CONCLUSION

IS is a condition that has not been well-studied. Clinical implications are not clear, and thus treatment guidelines are lacking. Particularly in patients who are asymptomatic, the need for treatment is controversial. This report describes an incidental finding of IS in an asymptomatic patient with a history of HBV and leukopenia. This patient was managed without antibiotics and was followed carefully. He remained asymptomatic 7 wk after diagnosis. When evaluating immunocompromised patients, including those with HIV or viral hepatitis, one should consider the possibility of IS colonization, particularly in patients with gastrointestinal symptoms. This case highlights the feasibility and success of conservative management without use of antibiotic therapy in asymptomatic IS. Additionally, close monitoring with collaboration and shared decision-making between gastroenterologists and infectious disease specialists for asymptomatic IS was beneficial. Future research is needed to evaluate the impact of *Brachyspira* colonization of the gastrointestinal tract and to establish recommendations for treatment and follow-up, specifically in asymptomatic patients.

FOOTNOTES

Author contributions: Mizrahi J and Clores M performed conceptualization; Mizrahi J, Yee EU, and Clores M performed patient care; Novotny S performed literature review; Novotny S, Mizrahi J, and Yee EU wrote the original manuscript draft; Novotny S, Mizrahi J, Yee EU, and Clores M performed review and editing of the manuscript; Mizrahi J and Clores M performed supervision of the manuscript; All authors have read and agreed to this version of the manuscript.

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Steps taken to fight the COVID-19 pandemic at the grassroots level of rural India: Experience of a community physician

Ankit Chandra

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Abstract

Coronavirus disease (COVID-19) was first identified in Wuhan, China and then rapidly spread all over the world. The World Health Organization declared a pandemic on March 11, 2020 as a result of COVID-19. As it has caused substantial morbidity and mortality, most countries took several measures to control its spread, including India. As of now, India has witnessed three major waves of COVID-19. Several measures like nationwide lockdown, active case finding, contact tracing, home isolation, and vaccination were taken. There was involvement of multiple sectors (including private and government) and community participation. During the pandemic, I was posted at a primary health centre in a rural setting in India. A rural primary care setting has its own limitations. All the steps taken had several challenges at the grassroots level. Therefore, through this article, I highlighted the challenges faced during the implementation of steps taken to battle the pandemic and the outcome.

Key Words: Lockdown; SARS-CoV-2; Challenges; Strategy; Village

Core Tip: Numerous steps were taken in response to the coronavirus disease (COVID-19) pandemic including nationwide lockdown, active case finding, contact tracing, screening, home isolation, transfer of patients to dedicated care centres, vaccination, and activities to generate disease awareness. Several challenges in the implementation of these steps at the grassroots level were overcome with the involvement of multiple governmental and private sectors. The grassroots workers were the backbone in the management of the pandemic in rural areas. Despite taking various steps to combat COVID-19, we may not have been able to prevent or control the waves of COVID-19.

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INTRODUCTION

In December 2019, the World Health Organization learned of reports of a cluster of 'viral pneumonia cases of unknown origin' in Wuhan city, People's Republic of China[1]. On January 30, 2020, the Director-General of the World Health Organization declared the outbreak of a novel coronavirus that was named 2019-nCoV at the time as a Public Health Emergency of International Concern[2]. On the same day in India, Kerala state's Ministry of Health and Family Welfare reported the first case of 2019-nCoV from a student who had travelled from Wuhan city[3]. On March 11, 2020, the World Health Organization declared a pandemic as a result of coronavirus disease 2019 (COVID-19)[4]. On the same day, the Government of India requested state governments to invoke the 'Epidemic Act 1897' to address the COVID-19 emergency, and it was made a notifiable disease[5]. As of now, India has witnessed three major waves of COVID-19 in July to October 2020, April to May 2021, and January to February 2022[6]. India had a total of 44658365 COVID-19 positive cases and 530479 deaths (1%) through November 4, 2022[7].

The Indian health system in rural communities consists of a three-tier system with three levels of health care facilities: Primary level [includes sub-centres/health and wellness centres, and primary health centres (PHCs)]; secondary level (includes community health centres); and tertiary level (includes district hospital and medical colleges). During the period of the COVID-19 pandemic, I was posted as a postgraduate trainee at a PHC in a rural setting of Haryana state (India). The workforce at the PHC includes a medical officer-in-charge, staff nurses, lab technician, pharmacist, health supervisors, and supporting staff for sanitation and security. This PHC is affiliated with an academic institute. Therefore, it has undergraduate and postgraduate students posted for training purposes. Under this PHC there are 12 sub-centres. A subcentre is the most peripheral contact point and has a workforce of two multipurpose health workers [male multipurpose health worker (MPW) and female MPW]. Every village has Accredited Social Health Activists (ASHAs) who are honorary volunteers from the community and act as a link between the community and the healthcare system. This PHC caters to a population of around 50000 residing in the 17 villages and has 47 ASHAs. The medical officer's role in COVID-19-related activities in a PHC were in planning and execution of active case detection and surveillance, contact tracing, monitoring/management of COVID-19-positive patients, and the vaccination program against COVID-19. I had the opportunity to closely see these steps taken and the challenges faced for the COVID-19 pandemic response at the grassroots level[8]. This article highlighted the issues faced along the journey and their outcomes.

STEPS TAKEN

Lockdown and its effect

In rapid response to the COVID-19 pandemic, one of the major steps that was taken by India was to implement a nationwide lockdown from March 24, 2020 to May 31, 2020[9]. This was prescribed by the National Disaster Management Authority of India in exercise of the powers under the Disaster Management Act of 2005[10]. It included travel restriction, shutting down educational institutes, offices, industries, shops, and places of worship, closure of hospitality, and restriction in gathering. The lockdown was released in a phased manner, starting on June 1, 2020[11]. Unlike the villages, a strict lockdown was enforced by the government in urban areas. The halt of public transport caused misery to many migrant daily wage workers who had come for crop harvesting and were stuck in rural Haryana. During the lockdown, most of the households had financial hardships as the economy in the rural setting is mostly informal and operates primarily with cash. Shops selling alcohol and tobacco were closed as they were non-essentials. People dependent on tobacco and alcohol had withdrawal symptoms and started purchasing them illegally. During the lockdown, we also saw an increase in the number of patients in primary health care due to the lockdown. PHCs attended many complicated cases, which were follow-up cases from tertiary health centres or that needed tertiary care.

Multisectoral coordination

During the pandemic, there was coordination with multiple sectors like the education department, police department, Ministry of women & child development, Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy, sanitation department, and local village council (panchayat). There was absolute cooperation and coordination between the government and private

health sectors. The police department coordinated the execution of various plans like lockdown and cluster containment. The police department also extended its support in maintaining law and order during the conduction of community surveys, contact tracing, and isolation of patients. School and college teachers (from the education department) and Anganwadi workers (Ministry of women & child development) were also involved in conducting the active case-finding survey on COVID-19.

Due to the shortage of isolation facilities, a few of the schools and colleges were converted to COVID-19 care centres that were managed by medical officers. During the management of the COVID-19 pandemic, the traditional medicine system of India, which is Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy, was also involved[12]. There was the distribution of ayurvedic medications in the community to boost immunity by the Ministry of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy. Yoga and Naturopathy were also promoted in the community for prevention and symptomatic management. Medical officers (Ayurveda) posted at the primary health care level worked in patient monitoring, contact tracing, and led the camps for COVID-19 testing and vaccination. For the first time, Ayurveda and Allopathy worked together at a mass level. Patients with influenza-like illness (ILI) also sought medical treatment from private labs, chemists, and non-government clinics. A list of these patients was maintained at these private facilities and was shared with the district administration to track and test these patients.

Community involvement

The community was involved in activities related to surveillance, restriction of movement, disinfection, and promoting COVID-19-appropriate behaviour. The community helped the administration by providing information related to any in-migration (travel from foreign regions) and cooperated during the symptomatic screening in the village. The village sarpanch (head of the locally elected village council) coordinated to prevent any public gathering in their village. To disinfect the public places in the villages, sodium hypochlorite solution was sprayed by workers of the panchayat (locally elected village council). Restrictions to enter the villages were made by the community to prevent virus spread. The people of the villages took the lead and guarded the entry and exit gates of their village. Occasionally free distribution of masks was led by community leaders and non-governmental organisations. We found that the people in the community preferred to wear a piece of cloth (household fabrics/scarf/towel/handkerchief) around their faces over a surgical mask (procedure mask) or N95 mask. This could be due to the poor availability of masks (surgical or N95 masks) or due to the unaffordability[13, 14]. This could also be due to the feeling of comfort in a piece of cloth, which can be easily recycled by washing[15]. In the community, mask hygiene and safe disposal of surgical masks and N95 mask was difficult[16].

COVID-19 screening

Initially, the negative news in the media scared the community and increased the stigma against COVID-19. Diagnosis of COVID-19 was considered a death sentence. Community members tended to hide the ILI symptoms and would not show up for COVID-19 testing. Symptomatic people testing negative for rapid antigen test were advised to take an RTPCR test[17]. There were numerous revisions done to the testing strategy based on the evolution of testing facilities and community transmission of infection[18]. The uptake of RTPCR tests was limited due to distant government facilities to perform the test and the high cost of RTPCR in limited private facilities. Hence, the government has made the RTPCR sample collection facility available at the PHC to increase the uptake of the RTPCR test. Samples were collected at the PHC and were transferred to the tertiary centres equipped with facilities to run the test. The problems faced were the spillage or contamination of samples and delay in reporting results. There were several mass screening camps organised for COVID-19 testing in the villages. Medical mobile units were also deployed to reach the people in the villages, this was an example of delivering health through the principle of equity.

Active case finding, contact tracing, and cluster containment

Microplanning was completed for the active case finding and a survey conducted by ASHAs, Anganwadi workers, MPWs, teachers, and volunteers from other departments. Large surveys were conducted in the villages where door-to-door screening for ILI symptoms or any history of recent travel to high-risk areas. If anyone was found positive for screening, then monitoring and testing for COVID-19 was done. For conducting these surveys several training sessions and planning were done. Contact tracing was another herculean task as it involves the enumeration of all the contacts of COVID-19-positive patients, monitoring of all the contacts for any development of symptoms and testing for COVID-19. It was challenging due to the stigma related to COVID-19 and higher social mixing in rural communities. We had to deal with all the queries and anxieties of the quarantined contacts. We had to convince them to be screened for COVID-19 and monitor them daily until the end of the quarantine period.

For the cluster of COVID-19 cases, there was the formation of a containment zone to avoid the spread of disease, which was accomplished by sealing the geographical area. In this area, daily house-to-house surveillance, contact tracing, monitoring of cases, and testing of symptomatic community members in

the containment zone was done by the MPWs, ASHAs, and Anganwadi workers. This cluster containment strategy to break the chain of transmission was activated by the district administration through their chief medical officer and deputy commissioner[19].

Isolation and quarantine

In the first wave, patients who tested positive were transferred to isolation centres (dedicated COVID-19 care centres or hospitals) by a special ambulance, and their contacts were quarantined. All the ambulances posted at PHCs were called back to the district hospital and worked for the transportation of COVID-19 patients. They were made available through a district control room. Few people perceived the isolation in the centres as an impingement on their autonomy and liberty. There was a belief that the services and quality of these centres were subpar[20].

The household of a COVID-19-positive patient was labelled with a warning sticker, which created anxiety among the villagers. This was a tough situation for the members of the household because of the stigma towards them. After a while, this practice was withdrawn[21]. As the number of positive cases rose, the healthcare system was overburdened. Then, the concept of home isolation for mild cases was introduced. We formed a team and visited all the positive cases and distributed a home-based kit (containing a biomedical waste bag, masks, bleaching powder, vitamin C and paracetamol tablets, and a pamphlet with self-care guidelines). For COVID-19-related waste generated at the household of a COVID-19-positive patient, we tried to safely collect and dispose at our PHC through the arrangement made with the help of a sarpanch/caregiver/health staff.

Involvement of ASHA

Similarly, like many health programmes in India, ASHAs were given training and were actively involved in COVID-19-related activities like active case finding, contact tracing, surveillance, and patient monitoring. Later, ASHAs were provided with a pulse oximeter to monitor the home isolated patients.

Disease awareness activities and promotion of COVID-19 appropriate behaviour

In the villages, the panchayat took the lead in promulgating COVID-19-related messages. Efforts were made to raise general awareness about COVID-19 among the individuals visiting PHC through posters, pamphlet distribution, videos, and health talks. ASHAs and MPWs were trained to provide health education in the community during their field visits.

At the national and state level of health promotion, there were various strategies used for health promotion, which were used in other national health programmes too. Caller tunes were used, and messages were printed on daily use items like milk and curd packets. Awareness was created through television, newspapers, and radio. Help from the entertainment industry was also taken to create related music and videos. There was the use of designer masks and caps and placement of posters at public places (hospitals, traffic signals, and bus stops). Awareness was generated not only by the government but also by the non-government sector. Most mobile applications changed their icon to a facemask, which created a daily reminder for people to wear a facemask. There were websites and digital applications that provided COVID-19-related information, national and state toll-free helplines, and other various helplines were started on social platforms like Telegram, WhatsApp, and Facebook.

Reporting and digital interventions

Reporting related to COVID-19 testing and the status of home isolation/quarantine was completed on a daily basis to several portals of the Ministry of Health and Family Welfare, Indian Council of Medical Research, Integrated Disease Surveillance Programme and other portals. Several communications and daily reporting were done through WhatsApp and emails. As daily reporting was done in multiple online portals and to various authorities, there was a huge amount of work related to data entry and reporting. On April 13, 2020, the government of India rolled out 'eSanjeevani OPD' to provide telemedicine services to patients isolated/quarantined at home. Unfortunately, this had poor coverage [22], which could be due to low digital literacy.

There were numerous meetings and trainings conducted at various levels. With the concept of digital meetings, we did save some time. A technological solution for contact tracing and syndromic mapping like Araogya Setu mobile application was not useful in a rural setting where the digital literacy is quite low.

The launch of the covid vaccine intelligence network (Co-WIN) application for vaccination was laudable. This application serves the function of registration, scheduling an appointment at their nearest location, and generating a COVID-19 vaccination certificate. The data entry operator posted at PHC and MPWs in the field had to enter the detail of each vaccine beneficiary into this application. Through this application, authorities can monitor the real-time vaccine status, coverage of the vaccine, and immediate adverse effects reported by a health facility.

Mass vaccination against COVID-19

For the first time, several vaccines were developed against a single disease, and all of them were

approved for emergency use in a short interval. India also developed and introduced its indigenous vaccine (Covaxin/BBV152) against COVID-19 in January 2021[23]. The majority of vaccinations were completed using Covishield and Covaxin vaccines. It was first given to healthcare workers from January 16, 2021 onwards. The vaccination was available for citizens who were aged ≥ 60 years and who were high risk from March 1, 2021 onwards. Other citizens were eligible for vaccination in phased manner (details in Figure 1)[24]. India became the first country to administer 1 billion doses of vaccine by the end of October 2021. This was done through conducting door-to-door vaccination (Har Ghar Dastak), mass vaccination camps/drives, and using the Co-WIN app. In the field, we did see vaccine hesitancy among the people. This hesitancy was reduced after the leaders and celebrities promoted the COVID-19 vaccination in our country. People also complied with vaccination against COVID-19 as it was made mandatory to produce vaccination certificates in several places for the purpose of travel, admission at events, and jobs.

Measures taken at the PHC

In our healthcare centre, we started screening all patients for ILI symptoms and high temperature using an infrared thermometer. A separate flu clinic was started to segregate the patients with flu symptoms, and daily reporting was done to the district. We started daily disinfection of the PHC with sodium hypochlorite solution. This caused chemical damage to the furniture of the health facility. Initially, there was a shortage of personal protective equipment (PPE). As the lockdown ended, the supply of PPE improved. We ran 24 h emergency care services and routine services at the PHC using PPE. We trained all the healthcare workers on the utilisation PPE and its safe disposal. Female staff faced difficulty in maintaining menstrual hygiene during the prolonged use of PPE. COVID-19-related waste generated at the field/subcentre was brought back to the PHC for safe disposal.

CHALLENGES FACED AND SOLUTIONS

At the grassroots level, we faced several challenges; some were managed and some were not. Initially many of the MPWs and ASHAs were hesitant to visit the households of any COVID-19-positive case. Many of the healthcare workers had comorbidities. The lead was taken by the medical officers and young healthcare workers in the field. Initially, to reduce the exposure we reduced the number of staff by half, and workdays were changed to alternate weeks. As the understanding of the disease improved, the involvement of the healthcare workers and the cooperation from the community increased. There were steps taken to protect all healthcare workers from COVID-19 such as the use of chemical prophylaxis (hydroxychloroquine tablets), PPE (gown, face shield, N95 mask, shoe cover, gloves, and head cover), and the introduction of the vaccines. Despite all these measures, the number of positive cases among healthcare workers rose substantially. This caused an acute shortage of staff. During this period, we learned task shifting and task sharing, which were done by training all the staff in COVID-19-related activities. Sometimes lab technicians and support staff were borrowed for a day from the neighbouring PHCs.

Later we trained our staff nurses in conducting rapid antigen tests and sample collection for RTPCR. The available trainees (students) at the PHC became involved in contact tracing and follow-up of home-isolated patients *via* telephone. Healthcare worker burnout and anxiety was real during the pandemic. To tackle this issue, a telephone helpline handled by a professional psychiatrist and psychologist was launched by the state government. This helpline also helped the staff, patients in isolation, and citizens to mitigate stress through online counselling. This was a commendable and well-appreciated initiative to provide psychosocial support. The government of India launched an insurance scheme for healthcare workers fighting COVID-19 (Pradhan Mantri Garib Kalyan Package) to provide comprehensive personal accident coverage of INR 50 Lakh (USD 60446). The healthcare workers included community healthcare workers (ASHAs, MPWs), hospital staff (doctors, nurses, paramedics, technicians), and supporting staff of sanitation and helpers. This scheme was extended to private healthcare workers who may have been in direct contact or at risk of COVID-19[25].

After a few attacks of healthcare workers in India, there was the promulgation of an ordinance to amend the Epidemic Diseases Act of India, which was approved on April 22, 2020 to protect the health workers and property including their living/working premises against violence[26]. To boost the morale of healthcare workers, they received appreciation from the community, national leaders, and compliments from the media.

During community surveillance, we did receive a few false alarms raised by people. We started verifying the calls by the MPWs and ASHA. In a few areas, we did face some resistance to active case-finding surveys from the community. Help from the police and sarpanch were required to resolve the issues. Contact tracing and active case finding were highly resource-demanding activities, which were not useful in the later phase of the pandemic as community transmission had occurred. Despite knowing the fact that a substantial proportion of COVID-19 cases are asymptomatic[27], we continued the practice of symptomatic screening and contact tracing for a very long time. Moreover, the number of symptomatic cases during the peak of the COVID-19 wave were overwhelming for our healthcare

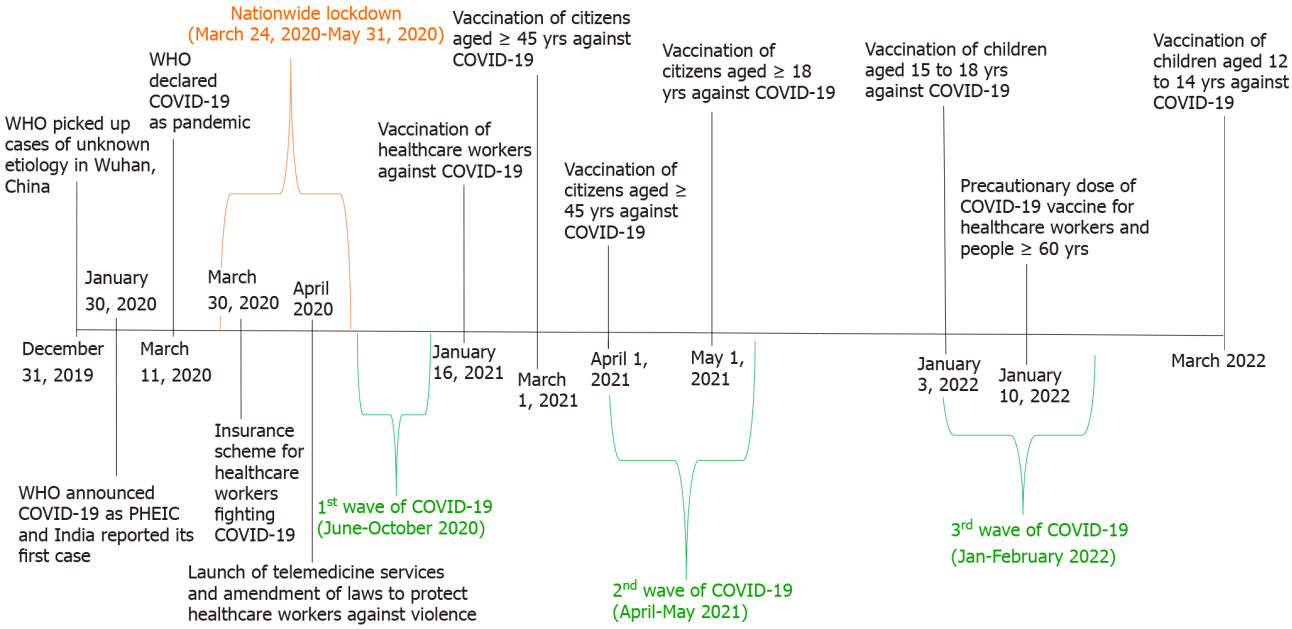

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Figure 1 Timeline of events that occurred during the coronavirus disease 2019 pandemic in India. COVID-19: Coronavirus disease 2019; PHEIC: Public Health Emergency of International Concern; WHO: World Health Organization.

workers to trace.

Home isolation also had its own challenges. In the villages, isolating a homemaker was a challenging task as women in villages are primarily involved in taking care of children and livestock. Isolating them for 1-2 wk was a major concern for their children and livestock, which are solely dependent on them. Furthermore, it was difficult to keep farmers away from their farmland because they needed to go to the field daily for irrigation and crop management. Thus, we asked for help from the village sarpanch, the patient's neighbours, or relatives to provide food and basic needs to the dependents. Our MPWs also frequently visited the patient's house to check on the dependent's health. Not only that, but a daily phone call was made at the PHC to check the patient, and their family's daily health status was updated over the phone. Home isolation was also challenging in cases where a patient did not have a caregiver, did not have a separate toilet in the house, had mental illness, or was experiencing homelessness. Hence, facility-based isolation was most suitable for these kinds of patients. Strict isolation was enforced by the head of the villages, police, and higher authorities as some of the quarantined patients and contacts were not following the isolation order.

We also found that a few patients provided the wrong address and mobile number during the COVID-19 testing, which caused problems in contact tracing later, if the result is positive. To tackle this, we started checking the address with a valid identity card at the time of testing. It was also found that COVID-19-positive patients were tested at multiple health facilities to get a negative report as soon as possible due to requirement to go back to work, double confirmation of diagnosis, a fear of COVID-19, and to confirm recovery. This also increased the burden on the healthcare system as their names were duplicated in the computer systems. Then, the government issued new guidelines stating that the retesting of COVID-19 was not required at the end of the isolation period. This solved the issue of multiple testing for COVID-19 and reduced the workload.

We faced a limitation of space at the PHC due to the creation of space for donning & doffing area for PPE as well as an isolated area for COVID-19 testing. Eventually, after a discussion with the higher authority, a separate COVID-19 testing booth was provided to overcome the limitation. With limited space, it was difficult to maintain physical distancing for the inpatient services and at the sites of COVID-19 testing and vaccination. We faced another challenge of training all the healthcare workers to use smartphones and various applications (like Co-WIN and WhatsApp), which were used for official communication/reporting, sharing the geo location, and vaccination details. For this, we conducted several training sessions for them at the PHC. In the initial period, there were various guidelines related to testing strategy, isolation and quarantine, clinical management of COVID-19, *etc.* issued by multiple institutions or organisations that created confusion among healthcare workers. Then there were national guidelines released, but all of these guidelines kept changing rapidly with advancing knowledge of the disease. We found it quite challenging to implement the newer guidelines in the community in a short period.

OUTCOME

The above steps taken were to break the chain of transmission and to assess the extent of COVID-19 infection in the community. Frequent estimation of seropositivity was a crucial indicator[28]. In our rural setting of Haryana state, the seropositivity during April to May 2021 (the initial phase of vaccination against COVID-19) was 59.3%[29]. The seropositivity among vaccinated and unvaccinated groups during July-August 2021 was 65.4% and 57.8%, respectively[29]. When we look at the national level, the seropositivity in India during May-June 2020 was found to be 0.73%[30], and it increased exponentially to 20.7% in the first wave and to 69.2% during the second wave of COVID-19[31]. Even during the nationwide lockdown there was a substantial increase in the test positivity rate[32].

The major steps included nationwide lockdown and COVID-19 vaccination. The nationwide lockdown may have been effective in enhancing the preparation (procurement of resources and preparedness for logistics and health infrastructure) and capacity building. The collateral consequences of lockdown were seen in a few unpublished research studies that were conducted in our setting and found a significant reduction in the coverage of routine immunisation for children and a substantial reduction in the utilisation of maternal care services during the lockdown. It also affected the follow-up and medication adherence in patients with tuberculosis or non-communicable diseases. Vaccination against COVID-19 may have been effective in reducing the severity of the disease in an unexposed person. Currently, the evidence suggests a pressing need to revise this COVID-19 vaccination strategy in India[33]. In the race of the COVID-19 vaccination drive, the single best vaccine against COVID-19 was not selected. Acknowledging the effectiveness of natural immunity was not accomplished. The COVID-19 pandemic had a short-term positive effect on the environment[34] and was a boon for promoting digital platforms/interventions. Mass-level promotion of health and hygiene could have a direct or indirect effect for controlling other infectious diseases.

CONCLUSION

Despite taking various steps to combat COVID-19, we may have not been able to prevent or control the waves of COVID-19. COVID-19 exposed the weaker links of our healthcare system, which provides us with an opportunity to strengthen the rural healthcare system for the future. The subject of public health in India is dealt by the state, therefore there could be variations across the states of India. However, the strategy remained the same across the whole of India, which was a five-fold strategy of test-track-treat-vaccinate and COVID-19-appropriate behaviour. Perhaps a concurrent evaluation of each step/strategy would have provided better learning and understanding.

The COVID-19 pandemic affected all levels of healthcare. We witnessed the brink of the breakdown of the health system, especially during the second wave when there were shortages of medical oxygen and beds in hospitals. We faced several challenges but never stopped healthcare services and fieldwork. We saw a huge and active collaboration of multiple sectors to combat this disease. During the COVID-19 pandemic, we saw an approach involving an empowered community, and multisectoral coordination, which are said to be the pillars of primary health care. COVID-19 has taught numerous lessons to everyone.

Due to the selective focus on COVID-19-related activities, other routine healthcare services for maternal and child health and major illnesses like tuberculosis, and non-communicable diseases were affected. The pandemic affected physical health and other domains of health (psychosocial, spiritual). COVID-19 has also affected the education and financial system of our society. It does provide a picture of the extent a disease affects human life.

The current healthcare system of rural India was not adequate to contain the transmission of COVID-19 and was not able to manage the load of COVID-19 patients. There is a paramount need to strengthen the infrastructure, laboratory services, regional surveillance systems, medical care, and skilled workforce. Currently, the healthcare system in rural India is heavily dependent on grassroots workers like ASHAs and MPWs. During the pandemic, ASHAs played a pivotal role in the management COVID-19 pandemic in rural areas. Furthermore, improving the support and recognition of grassroots workers are needed.

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