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ECCMID 2021

EDITOR'S PICK

Gross, Histopathological, and Ultrastructural Features in Patients with COVID-19: A Literature Review

INTERVIEWS

Nicola Rose and Sarah Caddy, two internationally renowned microbiology and infectious diseases experts, provide insights into the COVID-19 vaccination programmes and discuss their research interests and recent publications.

Contents

"Summaries of stand-out poster presentations are included in this issue, covering topics such as the impact of inflammatory bowel disease on the risk of periprosthetic joint infection, the use of plasma-activated water to combat multidrug-resistant bacteria, and much more."

Spencer Gore, CEO

Editorial Board

VIEW IN FULL ←

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EMJ allows healthcare professionals to stay abreast of key advances and opinions across Europe.

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EMJ Microbiology & Infectious Diseases 2.1

Welcome

Dear Readers,

We are delighted to welcome you to this issue of *EMJ Microbiology and Infectious Diseases*. Our newest eJournal is packed with the latest developments in the discipline, including peer-reviewed articles and exclusive interviews with internationally renowned experts in the field. We are also excited to share with you our in-depth review of this year's virtual European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 9th–12th July 2021, alongside poster summaries from the 31st iteration of this world-leading event.

The articles contained within this journal span a broad range of topics and cover groundbreaking advances in the field. Our Editor's Pick for this issue is a piece by Hussain et al., titled 'Gross, Histopathological, and Ultrastructural Features in Patients with COVID-19: A Literature Review'. This fascinating paper explores the macro- and micropathological findings present in each organ system in people with COVID-19. Doshi et al. present an interesting case of an opportunistic fungal infection in an immunocompromised patient. This issue also includes a compelling report by Christopher et al., which discusses the sex-related discrepancies of COVID-19 personal protective equipment.

For those who were unable to attend ECCMID 2021, please enjoy our comprehensive review of this fantastic event. Despite being executed in a virtual environment, ECCMID seamlessly delivered high-quality content. Summaries of stand-out poster presentations are included in this issue, covering topics such as the impact of inflammatory bowel disease on the risk of periprosthetic joint infection, the use of plasma-activated water to combat multidrug-resistant bacteria, and much more.

We are grateful to our interviewees, Nicola Rose and Sarah Caddy, who spoke about their inspiring research in virology and immunology in the context of COVID-19, as well as the impact of the pandemic on vaccination programmes.

I would like to take this opportunity to thank the Editorial Board, authors, interviewees, and the EMJ publishing team for their contributions in providing the latest developments to healthcare professionals. We hope that *EMJ Microbiology and Infectious Diseases* will inspire new ideas and contribute to breakthroughs in the field.

Spencer Gore

Chief Executive Officer, EMG-Health

Foreword

Dear Readers,

Firstly, let me say that I hope you are keeping safe during these unprecedented times.

It is a great pleasure to introduce the latest issue of *EMJ Microbiology and Infectious Diseases*. As with the inaugural journal last year, you will find the most up-to-date developments from across the discipline contained within these pages. This is because, despite the ongoing COVID-19 pandemic, we are committed to our goal of disseminating expertly written, peer-reviewed articles and case reports.

The Editor's Pick for this publication is the article by Hussain et al., titled 'Gross, Histopathological, and Ultrastructural Features in Patients with COVID-19: A Literature Review'. Despite rapid effors to delineate the clinical course, prognostic markers, and complications of severe acute respiratory syndrome coronavirus-2, its pathophysiology is still relatively poorly understood. In this fascinating and timely study, the authors therefore summarise macro- and micropathological findings present in each organ system in patients with COVID-19.

Poster summaries from the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2021, held for the first time as an online event, are also contained within the journal and not to be missed. These cover relevant topics such as vaccination effectiveness against severe acute respiratory syndrome coronavirus-2 in healthcare practitioners, plasma-activated water as a potential non-pharmaceutical antibacterial and antibiofilm therapy against multidrug-resistant bacteria, and antibiotic stewardship attitudes and beliefs among frontline staff nurses. For those who were unable to attend the annual meeting or simply wish to be reminded of the great success of the 31st iteration of ECCMID, I highly recommend the Congress Review. This features groundbreaking research news from the meeting on the use of aspirin to reduce the risk of serious cardiovascular events in patients with pneumonia, the disappearance of influenza and respiratory syncytial virus during COVID-19, and the presence of antibiotic-resistant bacteria in dog food across Europe.

I would like to thank all the authors, contributors, Editorial Board members, and reviewers for their excellent work in creating the 2021 issue of *EMJ Microbiology and Infectious Diseases*. I hope that this journal will help to enhance your knowledge and I look forward to receiving more high-quality submissions from you in the future.

Rajeshwar Reddy Kasarla

Professor and Head, Microbiology Department, Universal College of Medical Sciences, Bhairahawa, Nepal

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EMJ 6.2

Symposium Review

Pioneering Best Practices in Atopic Dermatitis: Results from the Quality-of-Care Initiative

Poster Review

Late-Breaking Abstracts: Health Status Benefits of Mavacamten in Obstructive Hypertrophic Cardiomyopathy and the Modifying Effect of Ejection Fraction on the Therapeutic Benefit of Omecamtiv Mecarbil in Heart Failure

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Understanding the Impact of Non-Dystrophic Myotonia on Patients and Caregivers: Results from a Burden of Disease Healthcare Survey

Oxy-hydrogen Gas: The Rationale Behind Its Use as a Novel and Sustainable Treatment for COVID-19 and Other Respiratory Diseases

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Congress Review

Review of the 31st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2021

Location: ECCMID 2021

Date: 9th–12th July 2021

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:13-23. Congress Review.

THIS YEAR, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) adapted to the unprecedented challenges of COVID-19 by hosting their first online event. As a consequence, participants at the 31st iteration of this world-leading congress on infection were unable to visit Vienna, Austria, which was set to house ECCMID 2021. Rather than viewing the digital congress as an obligation, born of necessity during the pandemic, the ESCMID Committee instead transformed it into an opportunity. The virtual meeting meant that geography was not a barrier to attendance; at a time when open sharing of scientific information from across the world is more important than ever, the increased inclusivity, diversity, and accessibility at ECCMID 2021 was crucial.

Over the course of 4 days, ECCMID broadcast educational workshops, meet-the-expert sessions, symposia, open forums, pipeline corners, and much more. Presentations spanned across the discipline and included challenges of treating bacterial infections in elderly patients, novel interventions to reduce healthcare-associated infections, innovations in COVID-19 surveillance, the present and future state of influenza and severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) vaccination, biomarkers and predictors for COVID-19 outcome, and the potential of metagenomic sequencing as a first-line diagnostic tool.

ECCMID 2021 incorporated new and innovative 'fireplace discussions' with keynote speakers. These sessions provided an opportunity for leading experts in the field of clinical microbiology and infectious diseases to engage with early-career researchers and academics. For example, Michael Houghton, Li Ka Shing Professor of Virology and Director of the Li Ka Shing Applied Virology Institute, University of Alberta, Edmonton, Canada, spoke about the development of a vaccine against the hepatitis C virus. Herman Goossens,

"Rather than viewing the digital congress as an obligation, born of necessity during the pandemic, the ESCMID Committee instead transformed it into an opportunity."

Professor of Microbiology and Director of the Department of Clinical Pathology at the University Hospital, University of Antwerp, Belgium, gave a talk entitled 'Transcending European Clinical Research in Infectious Diseases Through Collaboration to Break Down Traditional Silos.' These lectures were imperative in promoting the development and exchange of knowledge that is essential for the entire global population.

The 2021 programme was also notable for featuring 5 days of pre-ECCMID content, which covered subjects such as antimicrobial resistance, COVID-19, diagnostic developments, and vaccines. Overall, this supplemented the usual congress with up to 50 additional sessions.

Although there could be no on-site Trainee Association of ESCMID (TAE) Day at this year's annual meeting, ECCMID successfully organised two separate online TAE sessions. The first of these focused on recent publications by ESCMID Young Scientist Members and the TAE Outstanding Awards. The second session allowed young scientists to consult with experienced moderators, in a round-table setting, about future career paths.

Several major awards were presented as part of ECCMID 2021. The ESCMID Young Investigator Awards for Research in Clinical Microbiology and Infectious Diseases were presented to Jason Trubiano, Department of Medicine (Austin Health), University of Melbourne, Australia, and Marit van Gils, Department of Medical Microbiology, Academic Medical Center, University of Amsterdam, the Netherlands. Trubiano and van Gils gave presentations entitled 'The Emerging Role of Antibiotic Allergy Programs in Antimicrobial Stewardship' and 'Lessons Learned from Antibodies Against SARS-CoV-2,' respectively. Furthermore, this year's recipient of the International Sepsis Forum (ISF) ESCMID Sepsis Award was Isis Ricaño Ponce, Radboud University Medical Center, Nijmegen, the Netherlands, who is part of a team integrating -omics technologies (e.g., proteomics, transcriptomics, and metabolomics) with clinical data in order to identify the molecular basis for sepsis heterogeneity. It is hoped that this will facilitate the diagnosis of sepsis and ultimately lead to faster treatment and improved patient outcomes.

The authors of several standout ePosters submitted to ECCMID 2021 have provided summaries of their research, which are shared in this issue of *EMJ Microbiology and Infectious Diseases*. These include a single-centre retrospective study on the impact of reducing the duration of surgical prophylaxis in cardiovascular surgery, evaluation of vaccination effectiveness against SARS-CoV-2 in healthcare professionals, and *Aspergillus* sensitisation in people with severe asthma. Our independent congress review also contains an overview of groundbreaking news stories such as the discovery of antibiotic-resistant bacteria in dog food across Europe, the use of aspirin to reduce the risk of cardiovascular events in individuals with pneumonia, and the increased prevalence of HIV among elderly populations.

Read on for our key scientific insights from ECCMID 2021. We look forward to being part of the international microbiology and infectious diseases community again, hopefully in-person, at next year's congress in Lisbon, Portugal. ■

ECCMID 2021 REVIEWED →

Antibiotic Resistance Could Be Caused by Commonly Prescribed Classes of Drugs

PROTON pump inhibitors, β-blockers, and antimetabolites, commonly prescribed classes of drugs, could cause antibiotic resistance, especially in the *Enterobacteriaceae* family, despite the fact that they are not antibiotics. A new observational study presented on 10th July at ECCMID 2021 stated these antibiotic-resistant bacterial infections could cause extended hospital stays and lead to risk of increased mortality rates.

Bacterial antibiotic resistance is often associated with over-prescription and regular exposure to antibiotics. However, it has been noted that patients diagnosed, and admitted to the hospital, with drug-resistant bacteria show no common recognisable risk factors. Previous studies have identified that commonly used non-antimicrobial drugs (NAMD) play a significant role in antibiotic resistance because of the consequential effect on gut microbiome bacterial composition. The aim of this research was to address the role of NAMD use as a risk factor for infection with antibiotic-resistant bacteria.

The researchers from Tel Aviv Medical Center hospital, Israel, examined data from 1,807 patients diagnosed with upper urinary tract infection, and a positive urine or blood culture of Enterobacteriaceae, who were admitted in the hospital over a period of >2 years (from 1st January 2017 to 18th April 2019). The researchers then acquired the electronic medical records regarding the previous and current use of 19 NAMDs. The results showed that antimicrobial drug-resistant organisms were found in over half of the collected patient samples (n=944/1,807). Furthermore, approximately one-quarter of samples (n=431/1,807) revealed multi-drug-resistant organisms that were resistant to three or more antibiotic classes.

Interestingly, NAMDs such as selective serotonin reuptake inhibitors for depression symptoms, antipsychotics drugs for mental health, proton pump inhibitors for the reduction of stomach acid, β-blockers for conditions associated with heart problems, and antimetabolites for the treatment of cancer and other inflammatory diseases were all linked to increased antibiotic resistance. Additionally, antimetabolites emerged as the group of drugs with the highest influence on antibiotic resistance.

"Our findings highlight the importance of non-antimicrobial drug exposure as a risk factor for antibiotic resistance," stated lead author Meital Elbaz, Tel Aviv Medical Center. "We urgently need larger studies with more drug classes to confirm the discovery and to clarify the biological link between common prescription drugs and antibiotic resistance." ■

Reduction in Antibiotic Outpatient Prescriptions Has Been Noted Across the USA

RESEARCH carried out across more than 1,200 clinics in the USA revealed that the prescribing trends for antibiotics has reduced by 4% per year. The study was presented on 10th July 2021 at ECCMID 2021. There are >2.8 million antibiotic-resistant infections occurring every year in the USA, leading to up to 35,000 deaths and 20 billion USD in healthcare costs per year.

Over 9 million veterans receive outpatient care via the Veterans Affairs (VA) facilities, which covers >1,200 outpatient clinics. According to the researchers, this declining trend of antibiotic prescriptions is due to the antibiotic stewardship programmes executed by the Veterans Health Administration (VHA) health system, which began in 2014. According to the World Health Organization (WHO), infections such as pneumonia, tuberculosis, sepsis, gonorrhoea, and food-borne diseases have become difficult to treat as antibiotics become less effective. Shockingly, 266 million courses of antibiotics are prescribed to outpatients in the USA. In 2011, the VHA initiated a National Antimicrobial Stewardship Task Force (ASTF) to help control execution of antibiotic stewardship programmes in the VA and by 2014, the VHA demanded that all of the associated hospitals establish antibiotic stewardship programmes.

To verify the prescribing trend, the researchers of this study examined the data from VA pharmacies by reviewing the patterns in dispensed antibiotic prescriptions in VA outpatient clinics from 2011 to 2018. Furthermore, they calculated the number of days of therapy (DOT) per 100 outpatient visits for all antibiotics annually and, specifically, the five commonly used antibiotics: doxycycline, azithromycin, amoxicillin/clavulanate, ciprofloxacin, and sulfamethoxazole/trimethoprim.

The results showed that over the period of 8 years, the overall antibiotic prescriptions dispensed declined by an average of 3.9% per year, from 39.6 DOT per 100 visits in 2011 compared with 29.4 DOT per 100 visits in 2018. Additionally, the use of sulfamethoxazole/trimethoprim, commonly used for urinary tract infections, decreased significantly by 7% per year. However, outpatient prescriptions for doxycycline, azithromycin, and amoxicillin/clavulanate between 2011 and 2018 remained the same.

"Use of these three commonly prescribed antibiotics remains high and may be an appropriate target for antibiotic stewardship programmes in the VA to further reduce inappropriate outpatient prescribing," commented Haley Appaneal, Providence Veterans Affairs Medical Center, Providence, Rhode Island, USA. "It might also help combat resistance if national guidelines took stewardship principles into account when making disease-specific recommendations for antibiotic use." ■

"...the use of sulfamethoxazole/trimethoprim, commonly used for urinary tract infections, decreased significantly by 7% per year."

Newly Diagnosed HIV Shows Presence of Risk in Elderly

PREVALENCE of HIV in elderly populations is on the rise; approximately 50% of Americans diagnosed with HIV in 2018 were ≥50 years old. Despite increased awareness, new treatments, and early testing, this life-threatening epidemic still claims millions of lives each year. A common misconception is that older people are not at risk of HIV, which is untrue. Garcia Carcus, from the Central University Hospital of Asturias, Oviedo, Spain, shared the results of a case study in an 83-year-old male with newly diagnosed HIV and explained how "we must debunk beliefs among healthcare professionals that older adults are not sexually active or use drugs" and can also contract this deadly disease.

Carcus examined the medical history of this elderly patient at ECCMID 2021, 9th–12th July 2021. The patient had a history of heart problems and procedures, including an aortic valve replacement in 2013. He was admitted to a hospital in Spain in July 2019 for a month-long fever and weight loss. Blood tests showed that the patient had low iron, low levels of white blood cells, and impaired kidney function. Moreover, the blood cultures were negative for life-threatening blood infections.

Although the blood tests did not expose any worrying conditions, the imaging of the heart told a different story, showing that the valve replacement was not working and revealing a thickening of small veins characteristic of endocarditis. The doctors prescribed the patient an 8-week course of antibiotics; however, when returning home, the patient's fever continued to worsen.

Finally, the patient's condition required urgent medical attention and he was rushed to the emergency department. The medical staff tested the 83-year-old male for HIV amongst other sexually transmitted infections and the results were positive. He had a very high HIV viral load (180,564 copies/mL) and a CD4 count of 182, indicative of AIDS. This patient is one of the oldest to be newly diagnosed with HIV; luckily, with antiretroviral treatment, his HIV viral load has decreased, and he recently celebrated his 85th birthday.

Interestingly, the doctors believed he contracted the virus at around 70 years old; however, he denied having relations with anyone other than his wife. It is unclear when or how he got HIV, but importantly, as Carcus concluded: "This case serves as a reminder that the elderly are not immune to HIV infection." Overall, this case report highlights the importance of educating older patients about the risk of unprotected sex and the importance of testing all age groups, including octogenarians. ■

"In adults, the incidence of influenza A and influenza B was 11.5% and 13.1%, respectively, in 2019–2020. This was strikingly reduced to 0% in the later season."

Disappearance of Flu and Respiratory Syncytial Virus During COVID-19

COVID-19 rapidly spread from Wuhan, China, to the rest of the world, in the space of a few months. This infectious disease is responsible for millions of deaths around the globe and has led to lasting symptoms in some patients, such as difficulty breathing and the permanent loss of smell. COVID-19 has taken over the headlines in the past year but that is not all this disease has taken over.

A recent study presented at ECCMID 2021, 9th–12th July 2021, which took samples from Detroit Medical Center and Children's Hospital of Michigan, Detroit, Michigan, USA, aimed to unravel the impact of COVID-19 on the incidence of flu. Siri Sarvepalli, Wayne State University School of Medicine, Detroit, Michigan, and collaborators conducted PCR tests using samples from nasopharyngeal swabs to test for various infections, including severe acute respiratory syndrome coronavirus-2 and the respiratory syncytial virus (RSV). Additionally, swabs were taken from the throat to test for Group A Streptococcus, which frequently occurs after viral respiratory tract infections. The researchers drew comparisons between PCR test results for various infections between September 2019 to February 2020 and September 2020 to February 2021.

The findings showed a significant difference between the 2019–2020 season and the 2020–2021 season in the percentage of influenza and RSV infections in adults and children. In adults, the incidence of influenza A and influenza B were 11.5% and 13.1%, respectively, in 2019–2020. This was strikingly reduced to 0.0% in the later season. The same dramatic absence could be seen in RSV as incidence fell from 9.0% to 0.0%. This stark difference was mirrored in children: 20.2% to 0.0% incidence in influenza B. Interestingly, the prevalence of other respiratory viruses, such as human metapneumovirus, had also decreased. These interesting results were apparent across 42 medical centres in the USA, further confirming the reliability of the data.

The reason for the striking absence of the flu could be due to increased handwashing, social distance measures, and mask-wearing that has occurred during the pandemic. These simple yet effective techniques could be keeping other respiratory viruses at bay. Further to this, as seen in the swine flu pandemic, COVID-19 could be interfering with the ability of other viruses to infect individuals via viral interference. The paper concludes that flu cases are likely to rise again once the pandemic is over, but continuing hand-washing and other measures might keep the number permanently low in the future. ■

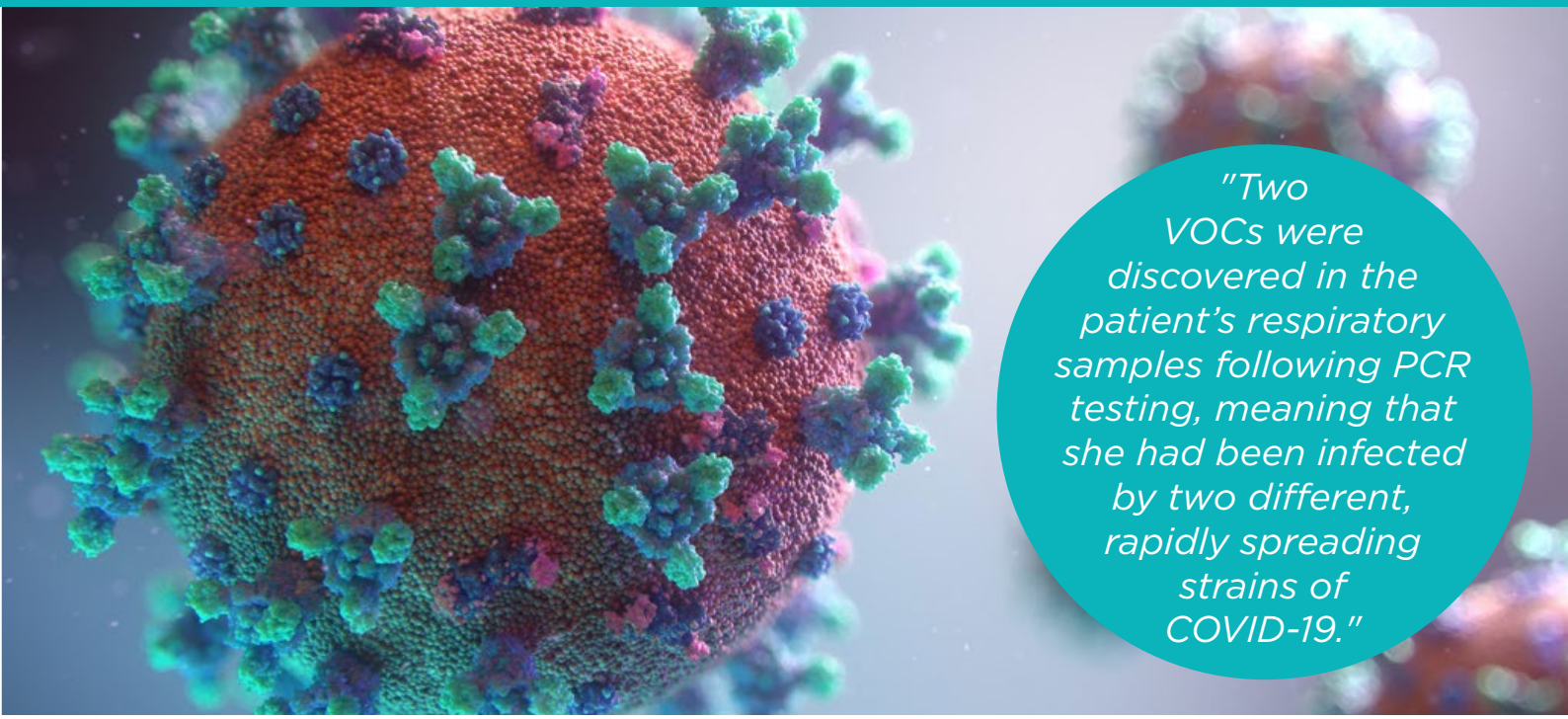
Aspirin Shown Effective at Reducing Risk of Cardiovascular Events in Patients with Pneumonia

"This study provides supporting evidence that aspirin use is associated with reduced ischaemic events after pneumonia in a primary care setting."

BRINGING forward findings relating to the interaction between cardiovascular complications and pneumonia, new research presented at ECCMID 2021 revealed that aspirin has the potential to reduce the risk of events such as ischaemic stroke and myocardial infarction (MI) in patients with this condition. The study, conducted by Fergus Hamilton and colleagues at the University of Bristol, UK, investigated the role aspirin might play in primary care settings.

Selection of the participant cohort was conducted from 48,743 patients with pneumonia over the age of 50 years, sourced from the Clinical Practice Research Datalink (CPRD). Primary outcome was defined as occurrence of both ischaemic stroke and MI, and secondary outcome was one of these events happening individually. Researchers uncovered that in the 8,099 aspirin users identified, matched with 8,099 non-users, there was a 36% lower risk of the primary outcome. Aspirin use was also associated with reduced risk of both secondary outcomes: 30% lower for ischaemic stroke and 54% for MI.

The large sample size allowed investigators to draw strong conclusions about the effective drug profile of aspirin from their findings. The researchers ultimately stated: "This study provides supporting evidence that aspirin use is associated with reduced ischaemic events after pneumonia in a primary care setting." In addition, the authors hinted at the implications of their work: "This drug may have a future clinical role in preventing this important complication." Hamilton went on to highlight the importance of their findings: "This research really paves the foundation for a clinical trial of aspirin in pneumonia, which remains the most common reason for admission to hospital in many countries." ■



Woman Infected with Two COVID-19 Variants Simultaneously

COVID-19 has impacted the world since its outbreak in Wuhan, China, in December 2019. The ability of the severe acute respiratory syndrome coronavirus-2 to rapidly mutate has seen the development of multiple variants, making COVID-19 increasingly difficult for the population to overcome. Researchers at the ECCMID 2021 presented a case of a 90-year-old woman with an unremarkable medical history, who was simultaneously infected with two COVID-19 variants of concern (VOC).

The patient, who had not been vaccinated against COVID-19, was admitted to the Onze Lieve Vrouwziekenhuis (OLV) Hospital, Aalst, Belgium, on 3rd March 2021 following repeated falls. She tested positive for COVID-19 and experienced no initial respiratory abnormalities. Her condition began to worsen, and she died 5 days later following the rapid development of respiratory symptoms and distress.

Two VOCs were discovered in the patient's respiratory samples following PCR testing, meaning that she had been infected by two different, rapidly spreading strains of COVID-19. The α strain (B.1.1.7) originated in Kent, UK, and the β strain (B.1.351) from South Africa. A second PCR test confirmed the presence of both strains through whole genome sequencing and S-gene sequencing techniques.

As this was one of the first cases of simultaneous infection involving two VOCs, Anne Vankeerberghen, lead author and molecular biologist at OLV Hospital explained: "Both these variants were circulating in Belgium at the time, so it is likely that the lady was co-infected with different viruses from two different people." Vankeerberghen added: "Whether the co-infection of the two variants of concern played a role in the fast deterioration of the patient is difficult to say." Previous reports by Brazilian scientists from January 2021 found that two people had also been simultaneously infected with two different strains of COVID-19, but this information is yet to be published in a scientific journal.

Vankeerberghen has posited that the global incidence of cases such as these are underestimated due to a lack of accessible testing options. She stressed the importance of being aware of co-infection rates, and encouraged scientists "to perform fast, easy, and cheap VOC-analysis by PCR on a large proportion of their positive samples, rather than just whole genome sequencing on a small proportion." ■

Will Current COVID-19 Vaccines Offer Sufficient Protection Against New Variants?

> *"Evidence showed that no reduction in protection was found against the α variant; however, there was a significant reduction against the β variant."*

FASCINATING evidence has emerged demonstrating the importance of receiving the second dose of the COVID-19 vaccine. This research was presented at ECCMID 2021, and also discussed the need to review and revise vaccines upon the development of new variants of concern (VOC). Nicole Schneiderhan-Marra and colleagues at the Natural and Medical Sciences Institute at the University of Tübingen, Germany, carried out a study to determine the levels of protection the current generation of vaccines provide against new mutated variants.

Due to the continuous mutation of the severe acute respiratory syndrome coronavirus-2, it is uncertain how effective current vaccines will be in providing protection against new variants. The protection that occurs following vaccination is determined by the levels of 'neutralising antibodies' present in the body. This specific group of antibodies act to defend the body and destroy the virus. Schneiderhan-Marra and colleagues assessed the levels of protection the vaccine provided for different strains through profiling antibodies produced following vaccination in the blood and saliva.

An existing assay, which measured the presence of severe acute respiratory syndrome coronavirus-2 antibodies in the blood, was altered to also detect VOC antibodies. Samples were collected from 23 people who had received two doses of the Pfizer–BioNTech vaccine. Control groups consisting of infected saliva donors, non-infected saliva donors, and infected blood donors were also included.

Analyses showed that the protection against COVID-19 increased significantly following two doses of the vaccine. The study compared the protection levels provided against the original 'wild-type' variant with that provided against the α and β variants of the virus. Evidence showed that no reduction in protection was found against the α variant; however, there was a significant reduction against the β variant. Schneiderhan-Marra explained: "This shows the importance of constantly updating vaccines to offer maximum protection against different strains of the virus."

With more variants emerging from the continuous mutation of the virus, scientists involved in this study have since adapted their assays to include new VOCs, including the dominant delta strain. Schneiderhan-Marra and colleagues are continuing to develop their research to monitor the emergence of new strains and their impact on current vaccine strategies. ■

Antibiotic-Resistant Bacteria Found in Dog Food Across Europe

"The close contact of humans with dogs and the commercialisation of the studied brands in different countries poses an international public health risk."

SURPRISING new evidence has revealed that raw dog food sold across Europe contains antibiotic-resistant bacteria. Presented at ECCMID 2021 on 10th July 2021, this novel research discovered multi-drug-resistant bacteria in the products, some of which are identical to 'superbugs' found in hospital patients across Europe. This information has identified raw dog food as an international public health risk that may be encouraging the spread of antibiotic-resistant bacteria.

The World Health Organization (WHO) have labelled antibiotic resistance as one of the greatest public health risks facing humanity, with approximately 700,000 people dying from drug-resistant infections every year. Ana Frietas, Carla Novais, Luísa Piexe, and colleagues from the UCIBIO Faculty of Pharmacy at the University of Porto, Portugal, studied different dog foods sold in supermarkets and pet shops for Enterococci bacteria. These opportunistic bacteria naturally live in the guts of humans and animals but can cause serious infections upon spreading to other parts of the body.

The study analysed 55 samples of dog food of different varieties. Fifty-four percent of samples contained Enterococci bacteria, >40% of which were resistant to erythromycin, tetracycline, streptomycin, gentamicin, quinupristin-dalfopristin, chloramphenicol, ampicillin, or ciprofloxacin. Twenty-three percent of the bacteria were also resistant to linezolid, which is a critical last-resort antibiotic used to treat serious infections.

Whilst all of the raw dog food samples contained antibiotic-resistant Enterococci, only three of the non-raw samples tested contained the multi-drug-resistant bacteria. Genetic sequencing of the bacteria in the raw samples found that they were genetically identical to bacteria in hospital patients around Europe, as well as bacteria found in farm animals and wastewater in the UK.

Scientists working on this study experimentally transferred the antibiotic-resistance genes found in the dog food samples to other bacteria with success, suggesting that this process could occur naturally with fatal consequences. Frietas explained: "The close contact of humans with dogs and the commercialisation of the studied brands in different countries poses an international public health risk." This research established raw dog food as a source of antibiotic-resistant bacteria that could spread to humans, and encourages pet owners to ensure they disinfect their hands after handling food or faeces. ■

Dogs May Be Passing Resistance to Vital Antibiotic to Owners

ALARMING reports have revealed the presence of the *mcr*-1 gene in four healthy humans and two pet dogs. This recent discovery was presented at ECCMID 2021 on 10th July 2021, and in both cases found the gene in both the dog and owner. The *mcr*-1 gene provides resistance to colistin, a last-resort antibiotic used to treat severe bacterial infections resistant to all other drugs.

Since its initial report in China in 2015, the *mcr*-1 gene has been reported in both humans and animals around the world. Due to its established resistance against colistin, there is a risk of this gene combining with drug-resistant bacteria, which would result in the development of an untreatable strain of infection. Juliana Menezes and colleagues at the Centre of Interdisciplinary Research in Animal Health at the University of Lisbon, Portugal, focused their study on whether household pets act as a reservoir of the *mcr*-1 gene, facilitating its spread throughout the community.

Scientists tested bacteria in faecal samples from humans and their pets for resistance to colistin. Of the 126 people and 102 cats and dogs, all of the humans and 61 of the pets were healthy. Pets considered 'unhealthy' were suffering from skin and soft tissue infections or urinary tract infections. Eight dogs, three of which were healthy, and four humans were found to be harbouring bacteria with the *mcr*-1 gene. All of these 12 samples were found to be resistant to antibiotics.

Genetic analysis of samples indicated that in one case, an *mcr*-1-positive dog with skin and soft tissue infections transmitted the gene to its human. Menezes explained: "While transmission in both directions is possible, it is thought that in this case the gene passed from dog to human." This evidence suggests that pets, particularly dogs, may harbour bacteria with the gene, promoting the overall spread of colistin resistance, which could have fatal consequences as a last-resort antibiotic. ■

"Due to its established resistance against colistin, there is a risk of this gene combining with drug-resistant bacteria, which would result in the development of an untreatable strain..."

Abstract Reviews

Sharing insights and updates from a selection of posters presented at the 31st European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), renowned clinicians and academics have provided these summaries of their fascinating and timely studies.

Is the Delayed Antibiotic Prescribing Strategy Effective for Reducing the Use of Antibiotics?

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Keywords: Antibacterial agents, antimicrobial stewardship, delayed antibiotic prescribing, primary health care.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:24-25. Abstract Review No. AR1.

BACKGROUND AND AIMS

Delayed prescription is recommended in international guidance for dealing with uncomplicated respiratory infections. Systematic reviews of delayed prescription, where the patient is advised to wait for at least the expected natural history of the illness before using the prescription, have concluded that the strategy is an effective way of reducing antibiotic use.[1] A 2017 Cochrane review including randomised clinical trials found that this strategy was associated with significantly decreased antibiotic use, as 31.0% of the cases admitted to taking the antibiotic, 33.9% when the prescription was patient-led, and 27.7% when this was collected at the centre.[2] However, the actual use of antibiotics in current practice might be higher than that reported in clinical trials. The authors assessed whether patients given a delayed prescription filled it or not and, if so, how many days the antibiotic was obtained after the index consultation.

MATERIALS AND METHODS

Patients were recruited from practices in urban primary care centres in Catalonia, Spain.

All the participating general practitioners were familiar with the delayed prescribing technique and routinely employed it in their practice. Eligible subjects were those of any age presenting with a sore throat with two Centor criteria and uncomplicated acute bronchitis from September 2018 until March 2020. The recruiting doctor issued an antibiotic prescription during the consultation but advised the patient to use it after 3 days in the case of sore throat and after 7 days for episodes of acute bronchitis, and only in the absence of spontaneous improvement, as suggested by the National Institute for Health and Care Excellence (NICE) guideline.[3] Participating general practitioners tracked the information and collected it in the electronic records, and registered whether the patients filled the prescription given and the dispensing date within the first 2 weeks after the index consultation.

RESULTS

A total of 126 patients were given a delayed antibiotic prescription, of which 82 cases corresponded to acute bronchitis. The mean age was 41.2 years (standard deviation: ±10.6 years), with 72 women (57.1%). The prescriptions were never filled in 52 cases (41.0%), but five patients took another antibiotic within the first 2 weeks. Out of 74 patients who did take the delayed prescription, 36 obtained the medication the same day of the visit (48.6%). Only 12 patients obtained the medication based on the instructions given by the doctors (16.2%).

CONCLUSION

The strategy of the delayed antibiotic prescribing resulted in a significant reduction in antibiotic use, but this reduction is not as high as the percentage observed in clinical trials, as only 4/10 were not taking antibiotics. In another study carried out in 13 European countries, 55% of the patients who were offered delayed antibiotic prescribing consumed an antibiotic during the study period.[4] Similarly, 30% started taking their delayed antibiotics on the day that they were prescribed. In the authors' study, this was even worse as only a small percentage of patients obtaining the medication adhered to the doctors' instructions. This study has several limitations. The authors cannot ensure that all the patients who filled the prescription had taken the medication, which is the most important limitation of this study. The method of delivering the delayed prescription to the patient may also influence how delayed prescriptions are used. Patients were not randomised; however, data on routine prescribing behaviour in everyday clinical practice can only be obtained through observational data, as in this study. ■

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Antibiotic Stewardship Attitudes and Beliefs Among Front-line Staff Nurses: Impact of Virtual Education in the COVID-19 Era

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Keywords: Antimicrobial stewardship (AS), education, nursing, virtual education.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:26-27. Abstract Review No. AR2.

BACKGROUND AND AIMS

Studies have shown that bedside nurses, who are vital members of the healthcare team, are often under-utilised in antimicrobial stewardship (AS) activities.[1] Several nursing responsibilities such as taking allergy history and obtaining cultures already overlap with AS activities and they can play a key role in promoting AS in resource-limited settings.[2,3] Nursing involvement in AS is required by The Joint Commission.[4] The AS team at the authors' institution (390-bed community hospital) wanted to educate front-line staff nurses regarding AS and incorporate some best practices in daily workflow. Due to COVID-19 restrictions, face-to-face education was not feasible. Therefore, this study was conducted to assess the baseline attitudes and beliefs of front-line staff nurses towards AS and see if a virtual education campaign consisting of newsletters and tip sheets would positively impact those beliefs and attitudes.

MATERIALS AND METHODS

The authors conducted a baseline survey in September 2020. Subsequently, they started distributing monthly newsletters via email. The nurse managers distributed emails and posted content on various AS topics such as obtaining an accurate allergy history, starting antibiotics promptly, using microbiology results to help guide decisions, and stopping therapy in cases of colonisation. A follow-up 6-month survey was conducted in March 2021 to assess the impact of the virtual education. Demographics and survey results were evaluated using X^2 and Mann–Whitney U test, as appropriate.

RESULTS

A total of 109 nurses responded to the baseline pre-survey in October 2020 compared to 64 nurses in March 2021. The baseline demographics were similar between the pre- and post-survey group in terms of education and primary role as a nurse. All adult specialties were well represented (intensive care units and medical and surgical nurses). Of those who responded in the pre-survey, 98% thought it was very important to obtain cultures accurately and understood the relationship between *Clostridium difficile* and antibiotics, and 96% of nurses surveyed agreed that nurses should participate in AS activities. The majority of nurses in the baseline survey (93%) believed that emerging resistance is an urgent threat to healthcare. The majority of pre-survey respondents listed knowledge gaps in microbiology (86%), antibiotics (84%), and scope of practice concerns (75%) as barriers to nurse participation.

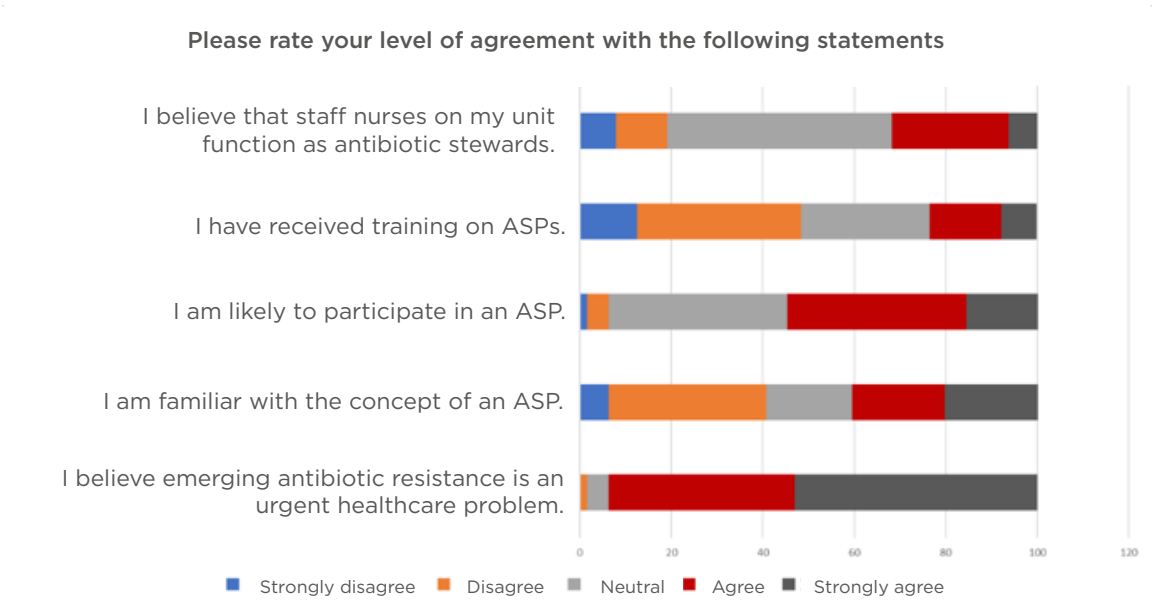

Figure 1: Nursing awareness of antimicrobial stewardship; post-survey percentage of respondants (N=64).
ASP: antibiotic stewardship programme.

The virtual education raised the familiarity with the AS programme, and more nurses in the follow-up survey said they were familiar with the concept of AS (32% pre- versus 41% post-education; p=0.02) and were likely to participate in AS (52% pre- versus 55% post-education; p=not significant) after the virtual education. Fewer nurses disagreed that staff nurses function as antibiotic stewards (36% pre- versus 19% post-education; p=0.0004) (Figure 1). However, no change was demonstrated in perceived barriers to nurse participation in AS activities despite education and no significant changes were noted in nursing attitudes towards various AS tasks pre- and post-education.

CONCLUSIONS

This study shows that the majority of nurses (>90%) are aware of the threat of antibiotic resistance and want to participate in AS activities. However, barriers to nursing involvement such as lack of knowledge, scope of practice concerns, and time constraints persist. Virtual education may increase nurse awareness of an AS, but needs to be supplemented with formal training and/or face-to-face education. When real-time, face-to-face instruction is not feasible, as in the COVID-19 era, virtual education via email, newsletters, and continuing medical education may supplement traditional methods to improve nursing participation in AS initiatives in an inpatient hospital setting. ■

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Be Conscious of *Cryptococcus neoformans*: Cryptococcal Meningoencephalitis and Cryptococcaemia with Unfavourable Outcome in a Patient with Myasthenia Gravis

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Cryptococcal meningoencephalitis, *Cryptococcus neoformans*, HIV-negative patient, myasthenia gravis.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:28-29. Abstract Review No. AR3.

BACKGROUND

Cryptococcus neoformans is an opportunistic fungal pathogen transmitted to humans by inhalation, mainly of infected bird droppings.[1,2] The infection caused by *C. neoformans* (cryptococcosis) is of global importance with significant attributable mortality, usually affecting the lungs and the central nervous system.[1,2] Patients with HIV infection are at high risk of developing cryptococcosis.[1,2] Nevertheless, non-HIV patient populations are also at risk. In HIV-negative individuals, predisposing risk factors include: solid organ transplant; corticosteroid therapy; chronic lung disease; and haematologic malignancies.[2] The authors report a case of cryptococcal meningoencephalitis and cryptococcaemia in a patient with myasthenia gravis, emphasising the need for clinical suspicion and timely laboratory diagnosis of cryptococcosis in HIV-negative patients.

CASE DESCRIPTION

A 74-year-old female patient was admitted to the high-dependency unit of the authors' hospital after being referred by a neurological clinic due to subacute meningitis. Her medical history was remarkable for myasthenia gravis, treated with mycophenolate mofetil and prednisolone for >1 year; allergic and asthmatic bronchitis; and Type 2 diabetes. She was initially diagnosed as having tuberculous meningitis with concomitant pulmonary tuberculosis, based on the clinical picture, chest CT, and compatible early findings of cerebrospinal fluid (CSF). She was receiving empiric quadruple anti-tuberculosis treatment in addition to common antimicrobials for 7 days. Despite the treatment, the patient was febrile at admission, with a reduced level of consciousness, and, due to hypoxia, she was intubated. The lumbar puncture performed in the emergency department showed 60 cells, protein 3.0 g/L, and glucose 0.61 mmol/L. The suspicion of *Cryptococcus* at the examination of CSF led to India-ink staining, which revealed capsulated yeast typical of *Cryptococcus*. *Cryptococcus* was also detected on the CSF by PCR, using the BIOFIRE® FILMARRAY® system (bioMérieux SA, Marcy-l'Étoile, France). The cryptococcal antigen test of both the CSF and blood specimens were positive. The patient received dual antifungal treatment: liposomal amphotericin B and fluconazole, since flucytosine was unavailable.[3,4] Yeast colonies isolated from CSF and blood cultures were identified as *C. neoformans*. Sensitivity testing showed resistance of the pathogenic strain to fluconazole. The patient deteriorated considerably in the following days and, due to the persistent isolation of fungus in CSF, intrathecal administration of amphotericin B was unsuccessfully performed.

DISCUSSION

This case highlights the need for early diagnosis of cryptococcosis in HIV-negative immunocompromised patients to prevent its fatal outcome. The value of screening by performing

India-ink stain on CSF specimens, even when biochemical and/or cellular parameters are normal, is unclear.[1,2] However, it may be of benefit in patients on immunosuppressive therapies, such as mycophenolate mofetil and corticosteroids.[2] It is worth mentioning, finally, that the emerging resistance of *Cryptococcus* strains to fluconazole makes treatment difficult in the absence of reliable alternatives.[3,4] ■

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A Single-Centre Retrospective Study on the Impact of Reducing Surgical Prophylaxis from 48 hours to 24 hours in Cardiovascular Surgery

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Antibiotic prophylaxis, cardiac surgery, cardiovascular surgery, coronary artery bypass graft, surgical prophylaxis.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:29-31. Abstract Review No. AR4.

BACKGROUND AND AIMS

There is limited evidence to guide duration of antibiotic prophylaxis in cardiovascular surgeries. While available evidence and guidelines generally recommend a duration of 24–48 hours,[1-4] there is limited comparative data to show whether 24-hour duration is as effective and safe as 48 hours. In November 2016, the surgical antibiotic prophylaxis (SAP) guidelines for cardiovascular surgeries at the authors' centre were updated. The prior guidelines recommended a 48-hour SAP with the use of intravenous cefazolin as a first-line agent and vancomycin for those with either severe β-lactam allergy or those colonised with methicillin-resistant *Staphylococcus aureus* (MRSA). In the updated guidelines, duration of SAP was reduced from 48 to 24 hours and patients colonised with MRSA received dual cover with both vancomycin and cefazolin instead of vancomycin monotherapy.[5] The authors conducted a retrospective study to review compliance to the updated guidelines and compare the incidence of surgical site infections (SSIs) pre- and post-implementation of the updated guidelines in order to evaluate the safety of the shortened SAP.

MATERIALS AND METHODS

A list of all patients undergoing cardiovascular surgery with sternotomy at the National Heart Centre between March 2016 and February 2019 was extracted from the hospital's electronic database. Every fourth patient in the list was included in the analysis. The patients were then divided into three groups: Group 1 (prior to the implementation of updated guidelines, from March to October 2016), Group 2 (following the implementation of updated guidelines, from October 2017 to May 2018), and Group 3 (June 2018 to February 2019).

Table 1: Evaluation of compliance to surgical antibiotic prophylaxis guidelines and incidence of surgical site infections.

	Group 1 (before new guideline implementation, March 2016 to October 2016) (n=149)	Group 2 (after new guideline implementation, October 2017 to May 2018) (n=184)	Group 3 (after new guideline implementation, June 2018 to February 2019) (n=176)
Appropriate choice of antibiotic prophylaxis, n (%)	145 (97.4)	178 (96.7)	169 (96.0)
Timely administration of surgical antibiotic prophylaxis,* n (%)	135 (90.6)	178 (96.7)	154 (87.5)
Prophylactic antibiotics stopped within 24 hours of surgery, n (%)	0 (0.0)	129 (70.1)	109 (61.9)
Prophylactic antibiotics stopped within 48 hours of surgery, n (%)	146 (98.0)	49 (26.6)	63 (35.8)
Prophylactic antibiotics stopped after 48 hours of surgery, n (%)	3 (2.0)	6 (3.3)	4 (2.3)
Surgical site infection within 90 days of surgery,† n (%)	7 (4.7)	5 (2.7)	9 (5.1)
Pathogens isolated from surgical site, n			
Coagulase-negative *Staphylococcus*	2	0	1
Methicillin-susceptible *Staphylococcus aureus*	2	2	4
Methicillin-resistant *Staphylococcus aureus*	1	0	0
Enterobacterales	3	1	5
Pseudomonas aeruginosa	1	0	0

*This is defined as follows: cefazolin administered within 30 minutes of incision and vancomycin administered at least 1 hour prior to incision.

†Some patients had >1 microorganism isolated and, in some cases, the diagnosis of surgical site infections was made based on clinical assessment (no microorganisms were isolated).

Patient demographics, MRSA colonisation status, drug allergy, antibiotic administration pre- and post-surgery, and the incidence of SSIs and corresponding culture data within 90 days of surgery were collected. Compliance to guidelines, in terms of choice, timing of administration, and duration of SAP, was assessed for all three groups. Both the incidence and microbiology of SSIs within 90 days of surgery were also evaluated.

RESULTS

A total of 509 patients were included in the study (Group 1: 149 patients; Group 2: 184 patients; and Group 3: 176 patients). Compliance to SAP and incidence of SSIs across the three groups are presented in Table 1. In general, the appropriate choice of antibiotic prophylaxis was consistently selected. SAP was administered in a timely fashion and highest rates of compliance were observed in Group 2, soon after the guidelines were implemented. With the implementation of the revised SAP, the proportion of patients on SAP for >24 hours decreased from 149 (100%) in Group 1 to 55 (29.9%) and 67 (38.1%) in Groups 2 and 3, respectively. Despite the reduction in duration of antibiotic prophylaxis from 48 hours to 24 hours, the incidence of SSIs did not increase. Among those who developed SSIs, the spectrum of causative pathogens was similar before and after implementation of the new guidelines.

CONCLUSION

Guideline implementation significantly reduced the duration of SAP from 48 hours to 24 hours in cardiovascular surgery at the authors' institution, with no increase in SSI rates. The appropriate class of antibiotics was consistently selected. However, there is a role for continual feedback to ensure that SAP is administered in a timely manner and not extended unnecessarily. ■

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Evaluation of Vaccination Effectiveness Against SARS-CoV-2 in Healthcare Professionals

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Disclosure: The authors have declared no conflicts of interest.

Keywords: Anti-SARS-CoV-2 spike antibodies, BNT162b2 mRNA COVID-19 vaccine, COVID-19 immunisation, Pfizer/BioNTech COVID-19 vaccine, SARS-CoV-2 IgG II Quant assay, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

Citation: EMJ Microbiol Infect Dis. 2021:2[1]:31-33. Abstract Review No. AR5.

BACKGROUND AND AIMS

The COVID-19 pandemic is an ongoing global pandemic that is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The latest data available (as of 28th June 2021) indicate that >181 million cases have been confirmed. With >3.92 million confirmed deaths attributed to COVID-19, it is one of the deadliest pandemics in history.[1] Prior to the pandemic, an established body of knowledge about the structure and function of pathogenic coronaviruses (causing SARS and Middle East respiratory syndrome) accelerated the development of various vaccine technologies during early 2020.[2] As a result, a wide range of COVID-19 vaccines utilise strategies that generate antibody response to the receptor-binding domain of the spike protein of the virus.[3] This ongoing widespread use of vaccines against SARS-CoV-2 has raised the important question on their ability to develop neutralising antibodies in levels sufficient to confer immunity to the COVID-19 infection. Moreover, the successful immunisation of healthcare professionals is of great importance and concern worldwide. The aim of this study was to evaluate the immunisation of healthcare professionals

Table 1: Serum IgG levels in naïve participants and in participants with previous exposure to severe acute respiratory syndrome coronavirus-2.

AU/mL	Naïve	With previous illness	Total
500–1,000	3	0	3
1,000–5,000	16	1	17
5,000–10,000	47	1	48
10,000–15,000	32	4	36
15,000–20,000	26	2	28
20,000–25,000	15	3	18
25,000–30,000	9	4	13
30,000–35,000	7	3	10
>35,000	20	5	25
Total	175	23	198

after a vaccination with the Pfizer/BioNTech BNT162b2 mRNA COVID-19 vaccine (Pfizer Inc., New York City, New York, USA; BioNTech SE, Mainz, Germany), using a quantitative measurement of IgG antibodies.

MATERIALS AND METHODS

A total of 198 health care professionals were included in the study. Blood samples were drawn approximately 2 weeks following the second dose of the vaccine, and the measurement of serum IgG antibodies against the spike domain of SARS-CoV-2 was performed using the SARS-CoV-2 IgG II Quant assay, a chemiluminescent microparticle immunoassay provided by Abbot Diagnostics (Abbot Laboratories, Abbot Park, Chicago, Illinois, USA).[4-6] The SPSS 25.0 (IBM, Armonk, New York, USA) statistical package was used and $p<0.05$ was considered statistically significant.

RESULTS

Of the 198 (32.8%) health workers, 65 were male and 133 were female (67.2%) aged 47.8±10.3 years old. All participants developed sufficient IgG titres in the blood, with the majority of them (56.6%) being between 5,000–20,000 AU/mL (normal range: 50–25,000 AU/mL). Only three out of 198 (1.5%) had relatively low IgG levels (500–1,000 AU/mL) but none below the detection limit (<50 AU/mL). Of the 198 participants, 23 (11.6%) reported previous exposure to the virus approximately 2–3 months before vaccination. Half of them (12/23) had very high serum IgG levels (>25,000 AU/mL), while only 36 out of 175 persons with no history of previous illness had similar IgG levels (52.2% versus 20.6%; p=0.045) (Table 1).

CONCLUSIONS

The BNT162b2 mRNA COVID-19 vaccine offers high protection against SARS-CoV-2, as it was 100% effective in producing anti-SARS-CoV-2 spike antibodies. Most healthcare professionals with previous history of COVID-19 disease developed very high immune response; probably, the second dose of the vaccine is not necessary to be administered to people with previous history of COVID-19 disease. ■

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Direct Linkage Between the Prevalence of Known Common Extra-intestinal Pathogenic *Escherichia coli* Sequence Types in UK Sewage to Those Causing *E. coli* Urinary Tract Infections and Sepsis

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Keywords: B2 phylotype, *Escherichia coli* (*E. coli*), sepsis, sequence types, sewage.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:33-35 Abstract Review No. AR6.

BACKGROUND AND AIMS

Escherichia coli are a hugely diverse group of bacteria that include harmless commensals as well as virulent pathogens. They are the leading cause of bacterial sepsis throughout Europe, which has more than doubled in prevalence in the last two decades. Currently, they are commonly classified by multilocus sequence typing into >10,000 sequence types (ST). Of these, only a tiny minority (0.06%), spearheaded by ST131, ST73, ST127, ST95, and ST69, cause the majority (>60%) of *E. coli* sepsis events throughout Europe and North America. The authors hypothesised that the rising rates of *E. coli* sepsis are due to rising human carriage rates of these virulent *E. coli* types in the UK community, and designed a study to test this hypothesis by analysing the prevalence of these types in UK regions with known disparate *E. coli* sepsis rates.

MATERIALS AND METHODS

Due to the vast amount of mechanical mixing in UK sewers and upon entry to wastewater treatment plants, homogenised human sewage is an ideal medium to use to measure community carriage prevalence of virulent *E. coli*. This is due to the fact that tens of thousands of human gut samples are normalised into a single sample, representing tens of thousands of individuals. Sewage was sampled (September 2019) on entry to six wastewater treatment plants in the UK located at the East and West ends of the M4 motorway corridor, locations chosen for low and high geographic *E. coli* sepsis rates, respectively. Three sites (Cardiff, Newport, and Bristol) were located at the west end of the motorway corridor and three sites (Reading, Marlow, and Longreach) were at the east end.

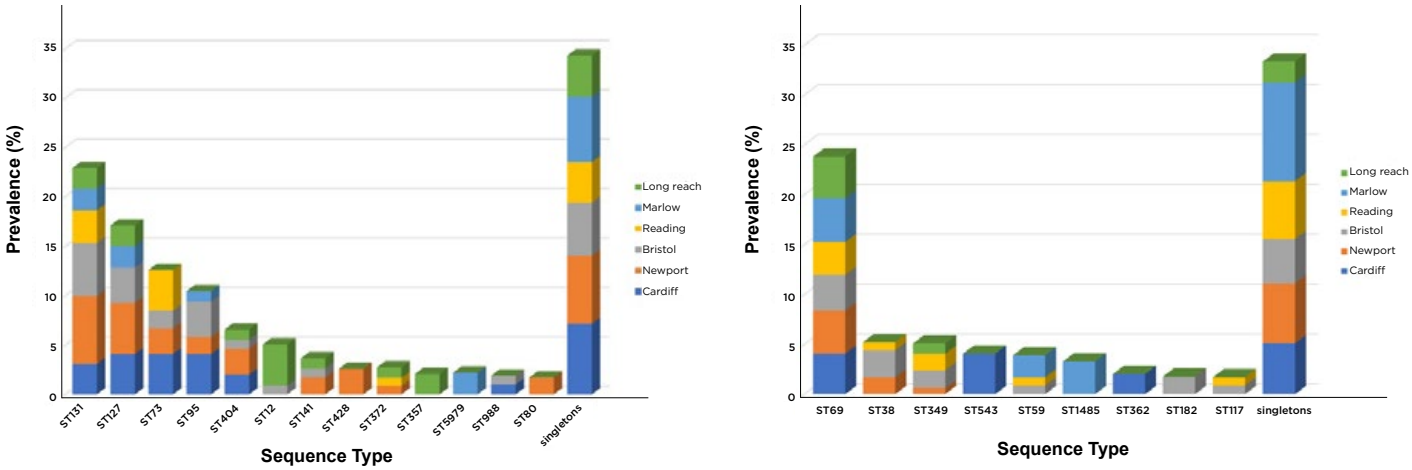

Figure 1: Chart indicating the percent prevalence of A) phylotype B2 *Escherichia coli* sequence types and B) phylotype D *E. coli* sequence types at each site in the UK. The most common sequence types corresponded to the sequence types that cause most UK sepsis events.
ST: sequence type.

At the same time, the authors sampled pooled sewage from nine sites in Dhaka, Bangladesh, to serve as controls because they had previously demonstrated very low carriage (0–1%) of virulent *E. coli* types in South Asia on several occasions.[1,2] Approximately 150 *E. coli* were randomly isolated (without antibiotic selection on Brilliance™ UTI Agar plates; Oxoid, Basingstoke, UK) from each sample, including triplicate and duplicate isolations from Newport (September and October 2019, respectively). Species identification was confirmed for 634 initial *E. coli* isolates and 410 replicate isolates from the Newport site by matrix-assisted laser desorption ionisation–time of flight mass spectrometry. Phylotypes were determined by the Clermont multiplex phylotype PCR[3] for all 1,044 isolates. STs were initially determined for all B2 and D isolates by Doumith ST PCR[4] and either confirmed or corrected by whole genome sequencing (WGS; n=224/634). Resistance genes and virulence genes were identified by *in silico* analysis of the WGS.

RESULTS

The prevalence of B2 phylotype *E. coli* was statistically different between M4 West and East sites (27% versus 15%; $p=9.2\times10^{-5}$) as well as between England and Wales (17% versus 29%; $p=2.5\times10^{-4}$), mirroring the *E. coli* sepsis rates. The most prevalent STs determined by WGS mirrored the most commonly identified *E. coli* types causing urinary tract infections and sepsis in the UK for both phylotype B2 and D (Figure 1). The Doumith PCR method gave poor results, misidentifying many isolates. Replicate samples indicated that sampling was statistically robust both within and between samples. Virulent *E. coli* STs from sewage mapped among bacteraemia isolates by core genome multilocus sequence typing and resistance/virulence gene analysis, including *fimH* typing.

CONCLUSIONS

The UK has a very high gut carriage rate of virulent extra-intestinal pathogenic *E. coli*, which is dramatically different to that of South Asia. The different prevalence in gut carriage between England and Wales provides a reasonable explanation of differing geographical *E. coli* sepsis rates and may also explain the overall rising *E. coli* sepsis rate for the UK over the last decade. ■

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Aspergillus Sensitisation in Patients with Severe Asthma

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Keywords: *Aspergillus* spp., asthma, basophil activation test, TARC.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:35-37. Abstract Review No. AR7.

BACKGROUND AND AIMS

Asthma is a chronic inflammatory disease of the respiratory tract and a serious global medical and social problem.[1] It is known that various aeroallergens play a key role in the triggering of asthma. Of special interest are thermo-tolerant fungi of the genus *Aspergillus*, which are able to reach the lower respiratory tract due to the small size of the spores. According to many authors, fungal sensitisation is associated with severe asthma course.[2,3] Several studies have shown that sensitisation to one or more fungal allergens is associated with lower lung function and higher incidence of exacerbations and hospitalisations.[4-6] Further characterisation of the phenotype of asthma with sensitisation to *Aspergillus* and the development of alternative therapy options are required to reduce the frequency of exacerbations and achieve better disease control.

The aim of the study was to assess the clinical and immunological characteristics of patients with severe asthma with *Aspergillus* sensitisation.

MATERIALS AND METHODS

The prospective study included 93 adult patients with severe asthma (four to five steps of therapy according to GINA 2021), with a median age of 52 years (males: 24).

The levels of thymus and activation regulated chemokine (R&D Systems, Minneapolis, Minnesota, USA), total IgE (Polignost, Saint Petersburg, Russia), and specific IgE (Alkor Bio, Saint Petersburg, Russia) to 10 allergens were determined in the blood serum by the enzyme immunoassay. Basophil activation test (BAT) with *Aspergillus fumigatus* allergen was performed *in vitro* using flow cytometry (Allergenicity kit; Beckman Coulter, Brea, California, USA). Basophils were identified by $CD3^{-}CRTH2^{+}$ marker and basophil activation by elevated expression of CD203c. The ISHAM 2013 criteria were used for *Aspergillus* sensitisation.[7]

RESULTS

Sensitisation to *Aspergillus* was detected in 35.4% patients with severe asthma. In the group of patients with *Aspergillus* sensitisation, the authors noted a lower score on the ACT questionnaire of 11.0 (10.0–16.0) versus 18.5 (13.5–21.0; p=0.000), lower FEV1 scores (57.0% [46.0–63.0] versus 72.5% [3.0–84.5]; p=0.001), and the FEV1/FVC ratio (68.0 [56.0-61.0] versus 73.7 [63.8–81.5]; p=0.025). Also, in patients with asthma with *Aspergillus* sensitisation, systemic corticosteroids were more often used to relieve severe exacerbations (36.4% versus 16.7%; p=0.043).

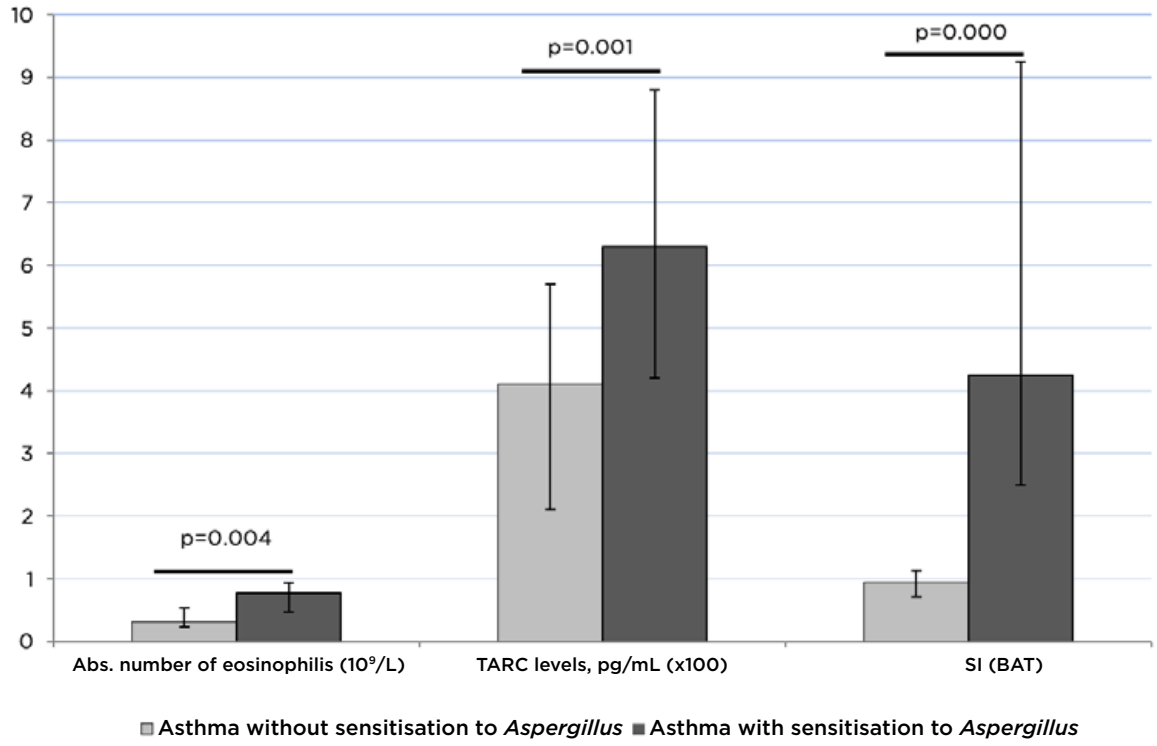

Figure 1: Immunological characteristics of patients with severe asthma with and without sensitisation to *Aspergillus*.

BAT: basophil activation test; SI: stimulation index; TARC: thymus and activation regulated chemokine.

In patients with asthma with *Aspergillus* sensitisation, the number of basophils activated by the *A. fumigatus* allergen and the stimulation index (SI) were significantly higher (9.9% [6.0–24.0] versus 3.6% [2.0–5.4], p=0.000; and 4.25% [2.49–9.30] versus 0.94% [0.75–1.1], p=0.000). Significant differences in the TARC content was obtained in patients with asthma with *Aspergillus* sensitisation (625.0 [418.4–875.0] pg/mL versus 406.0 [210.0–561.0] pg/mL; p=0.001). The revealed significant differences in the immunological characteristics of patients with severe asthma with sensitisation to *Aspergillus* and without sensitisation to *Aspergillus* are shown in Figure 1.

A negative correlation was found between TARC levels and a decrease in FEV1 (r=-0.70; p<0.05) and positive correlation with the absolute number of eosinophils (r=0.81; p<0.05) and sIgE level to *Aspergillus* (r=0.36; p<0.05).

CONCLUSIONS

The study revealed clinical and functional features of asthma with sensitisation to *Aspergillus spp.* as a separate phenotype of a disease with a more severe course and poor prognosis. The basophil activation test is an additional method for diagnosing *Aspergillus* sensitisation. The TARC concentration can serve as a biomarker of an active inflammatory response. ■

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Do Infections Start in the Gut? Patients with Inflammatory Bowel Disease Have a Higher Risk of Periprosthetic Joint Infection after Surgery

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Keywords: Infections, inflammatory bowel disease (IBD), periprosthetic joint infection (PJI), surgery, surgical infections, total joint arthroplasty (TJA).

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:37-38. Abstract Review No. AR8.

BACKGROUND AND AIMS

A large body of evidence is emerging to implicate that dysregulation of the gut microbiome (dysbiosis) increases the risk of surgical site infections. Gut dysbiosis is known to occur in patients with inflammatory bowel disease (IBD), allowing for translocation of bacteria across the inflamed and highly permeable intestinal mucosal wall. The null hypothesis was that IBD was not associated with increased risk of periprosthetic joint infection (PJI) after primary total hip and knee arthroplasty.

MATERIALS AND METHODS

A matched cohort study was designed. The primary endpoint was occurrence of PJI at 2 years. Secondary endpoints were aseptic revisions, as well as discharge to rehabilitation facility, complications up to 30 days, and readmission up to 90 days after total joint arthroplasty (TJA). ICD-9 and -10 codes were used to identify patients with IBD and the control cohort. A chart review was performed to confirm diagnosis of IBD. Using the institutional database, 152 patients with IBD were identified and matched (3:1) for age, sex, BMI, year of surgery, Charlson Comorbidity Index (CCI), and affected joint with 462 patients without IBD undergoing TJA.

RESULTS

The cumulative incidence of PJI was 4.55% among patients with IBD versus 1.32% among the control cohort (p=0.024). When univariable Cox regression was performed, a diagnosis of IBD was found to be an independent risk factor for PJI (hazard ratio: 3.61; 95% confidence interval: 1.21–10.74; p=0.021) and aseptic revisions (hazard ratio: 3.48; 95% confidence interval: 1.34–9.01; p=0.010). The rate of post-operative complications was also higher in patients with IBD.

CONCLUSION

Based on the findings of this study, it appears that patients with IBD are at higher risk for failure due to PJI or aseptic loosening after

primary TJA. The exact reason for this finding is not known but could be related to the bacterial translocation from the inflamed intestinal mucosa, the dysregulated inflammatory status of these patients, malnutrition, and potentially other factors. Some of the so-called aseptic failures could be as a result of infection that may have escaped detection and/or recognition.

Level of Evidence: Level II. ■

Plasma-Activated Water as a New Weapon Against Multidrug-Resistant Bacteria

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Keywords: Antibacterial, anti-biofilm, biofilms, ESKAPE, infectious diseases, multidrug resistance, plasma, plasma-activated water, therapeutic.

Citation: EMJ Microbiol Infect Dis. 2021;2[1]:38-39. Abstract Review No. AR9.

BACKGROUND AND AIMS

Multidrug-resistant (MDR) bacteria, or 'superbugs', pose an impending threat for human health around the world. Common infections are becoming more difficult to treat, with dire consequences for people needing invasive and life-saving surgery. Models predict 10 million annual deaths by 2050 as a result from infectious diseases caused by MDR bacteria.[1] Moreover, the majority of bacterial infections are associated with biofilms that, compared to planktonic forms, confer additional 10–1,000-fold tolerance to antimicrobial therapy.[2,3] Therefore, new therapeutic strategies are urgently needed to bypass MDR traits of bacteria.

The authors hypothesised that cold-plasma technology has the prospect as a non-pharmaceutical antibacterial and anti-biofilm therapy against MDR bacteria. Plasma discharged in water (plasma-activated water; PAW) forms reactive oxygen and nitrogen species,[4] which increase the conductivity and redox potential, and decreases the pH to create an antimicrobial environment capable of effectively killing bacteria. The study herein shows preliminary data on the biofilm-killing capacity of PAW and non-cytotoxicity in human cell culture.

MATERIALS AND METHODS

Five types of PAW (Plasmatreat GmbH, Steinhagen, Germany) were validated for antibacterial and anti-biofilm activity against the 'ESKAPE' pathogens *Escherichia coli*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus faecium*. The minimum inhibitory concentration of each PAW was determined following standard protocol (broth microdilution). Biofilms were formed over 48 hours before exposure to PAW for 30 minutes. Then, the anti-biofilm activity of PAW was measured by the AlamarBlue viability assay (Invitrogen, Thermo Fisher Scientific, Waltham, Massachusetts) and visualised by confocal microscopy with LIVE/DEAD BacLight staining (Invitrogen, Thermo Fisher Scientific). The viability of human keratinocytes after 30 minutes of PAW exposure was determined with the lactate dehydrogenase viability assay.

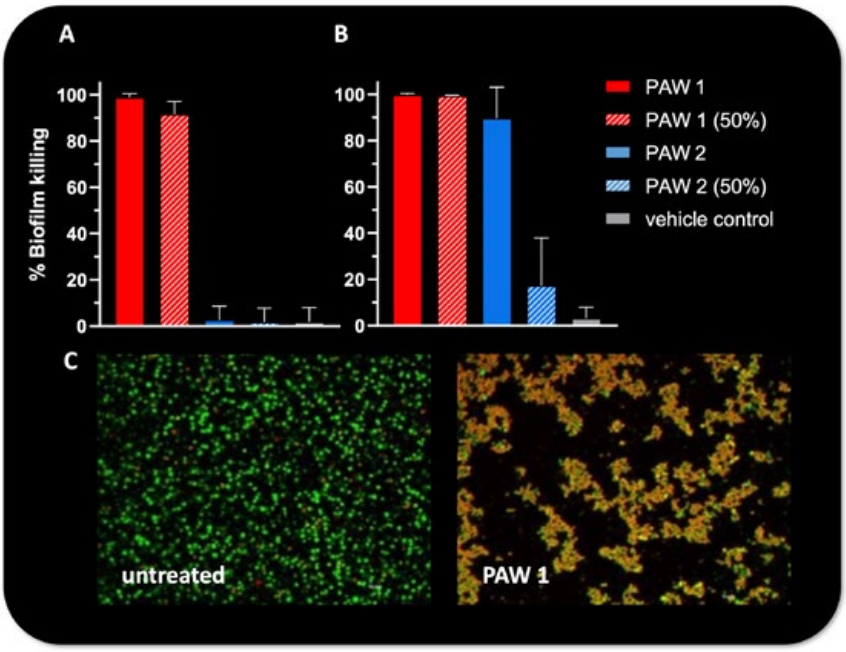

Figure 1: Biofilm killing of methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumannii* after plasma-activated water treatment.

Substantial biofilm killing of **A)** MRSA and **B)** *A. baumannii* after exposure to undiluted PAW and 50% diluted PAW in growth media. **C)** Biofilm killing of MRSA biofilms by PAW 1 compared to untreated control was visualised by confocal microscopy and LIVE/DEAD staining.*

*Green: viable bacteria; red: dead bacteria.

MRSA: methicillin-resistant *Staphylococcus aureus*; PAW: plasma-activated water.

RESULTS

Only PAW 1 and 2 effectively inhibited growth of all ESKAPE pathogens, with minimum inhibitory concentration values of 25% and 50% dilution, respectively. Substantial anti-biofilm activity was observed for PAW 1, showing 98% biofilm killing of MRSA (Figure 1A) and 99% biofilm killing of *A. baumannii* (Figure 1B), while PAW 2 was only effective against *A. baumannii* (90% biofilm killing). Results in MRSA biofilms were further confirmed by confocal microscopy. PAW 1 treatment substantially reduced the amount of MRSA biofilms and effectively killed bacteria, with the red cells indicating cell death (Figure 1C). Other PAW types were ineffective against planktonic and biofilm bacteria. All PAW types showed no toxicity in keratinocytes.

CONCLUSION

PAW exhibited high efficacy against MDR bacteria and biofilms of ESKAPE pathogens and no cytotoxicity in keratinocytes. These properties present specific types of PAW as a potential new treatment of microbial infections on human tissue, for example as an antibiotic-free lavage for surgical procedures or wounds. ■

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Interviews

In the following interviews, EMJ spoke to two leading microbiology and infectious diseases experts, covering topics such as their personal research interests, landmark publications, and the roll-out of COVID-19 vaccination programmes.

Featuring: Nicola Rose and Sarah Caddy.

Nicola Rose

Head, Virology Division, the National Institute for Biological Standards and Control (NIBSC), an Expert Centre of the UK's Medicines and Healthcare products Regulatory Agency (MHRA)

Q1

After your PhD in human molecular genetics, what drew you to studying retrovirology in your post-doctoral position at the University of Cambridge?

I was towards the end of writing my PhD thesis and looking at post-doctoral research positions but keeping my focus on the genetics field; there were some really interesting research posts available in excellent academic departments. However, one advert caught my eye, namely the post in Cambridge, studying pathologies associated with infection by a human retrovirus. When I had the opportunity to attend interview and hear more about the proposed project and the work in the research group, my interest was kindled. I made my mind up on the day to accept the post if it were offered. The group in Cambridge provided a superb environment for research. It brought me into contact with brilliant scientists and presented many opportunities for collaboration. My years in Cambridge provided a great stepping-stone to my subsequent position and proved that a PhD does not have to define a career path into any one field of study.

Q2

You have been at the National Institute for Biological Standards and Control (NIBSC) for 20 years and worked on different programmes. What has been the most rewarding project so far?

In the time I have been at NIBSC, I have been involved in various programmes of work,

each with its own rewarding outcomes. It is satisfying when projects bring together many international expert partners such as those who develop biological standards supporting disease diagnostics or vaccine development. Standards for Ebola and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in response to health emergencies, for example, required a rapid response from our teams. Our global partners assisted in sourcing biological materials and scientists at the NIBSC developed further materials using their own expertise. Laboratories across the world commit their time, resources, and skills to evaluate the samples we send them so that the most appropriate material can be proposed as a calibrated standard. These projects are truly international. Contributing to these programmes has a direct impact on patients and the public, the people who are relying on accurate diagnoses or availability of vaccines.

Q3

As you are involved in the quality assessment of vaccines, could you tell us the different standards that a vaccine has to meet in order to be approved for emergency use?

The regulatory agencies undertake thorough assessments of safety, efficacy, and quality data from clinical trials, which inform the decision whether to approve a vaccine for use.

If approved, the product itself is subject to testing before being used clinically. Vaccines are produced in large batches, each of which requires a series of checks before being placed on the market. The vaccine manufacturer performs an extensive range of tests on each batch. A condition of approval for use in an emergency might include assessment through an independent medicines control laboratory, such as the NIBSC in the UK. This assessment can include tests of the key parameters of the product, for example its identity and its potency as an independent verification of the biological quality for that batch of vaccine. Provided the laboratory assessment test results meet the specifications for the product as agreed through the regulatory approval process, and it is confirmed that the manufacturer's own tests have been satisfactorily completed, the individual batch can be certificated by the independent laboratory. Subsequently, the manufacturer completes its steps to release that batch to the market.

"My years in Cambridge provided a great stepping-stone to my subsequent position and proved that a PhD does not have to define a career path into any one field of study."

Q4 One of your research interests is RNA viruses, such as coronaviruses. How much did we already know about such viruses before the SARS-CoV-2 outbreak?

Coronaviruses are a family of viruses that, for the most part, cause mild to moderate illness in the upper respiratory tract of humans. The existence of the viruses has been known for decades and they have been studied widely. However, there is a large number of coronaviruses that circulate in animals and sometimes spill-over into humans and cause disease. To date three coronaviruses have been identified that cause serious, sometimes fatal, human disease. Severe acute respiratory syndrome coronavirus emerged in 2002, causing the first pandemic of the 21st century. The disease severely affected the Asia Pacific region and had a fatality rate of about 10% in the approximately 8,000 documented cases. The virus has not been seen clinically since 2004. Middle East respiratory syndrome, caused by the Middle East respiratory syndrome coronavirus, was first reported in 2012. There are still occasional outbreaks. and the virus has a case fatality rate of about 35%. SARS-CoV-2, the virus that causes COVID-19, is the third coronavirus to emerge this century and was declared a pandemic in 2020.

Q5 In May 2020 you were involved in producing a new biological reagent that helps develop accurate diagnostic tests for COVID-19. Could you tell us how this works?

It was clear in the early weeks of the SARS-CoV-2 outbreak that reagents to aid the diagnostics and research and development communities would be needed. We developed a non-infectious material that could be used as a positive control for systems to detect the presence of the virus. We also produced an antibody material from convalescent plasma that could be used as a positive control for the development and evaluation of serological assays that detect the presence of antibodies against SARS-CoV-2.

A positive control is included in tests by means of a sample that contains a known amount of the coronavirus genetic material. This acts as a confirmatory sample that assures the test is working correctly giving greater assurance for the operator that the test outcome is valid. The development of these materials was a prelude to the production of formally established calibrated standards and reference materials that the NIBSC produced later in 2020, with the input from international collaborating laboratories.

Q6

You wrote a commentary in June 2020 about the importance of biological standards in the development of diagnostic tests. How does this relate to the reagents that were made?

One of the reference standards indicated above is designed to provide robust, reliable molecular diagnostic assays. The NIBSC is one of a group of World Health Organization's (WHO) collaborating centres for biological standards and has a long-term programme to develop WHO International Standards, which are calibrated in international units. SARS-CoV-2 is just one of many viruses that have emerged over the years where working with the virus requires high biocontainment. To support laboratories undertaking diagnostics or developing vaccines, producing non-infectious materials that are safe to use is important. We had previously produced such standards for the measurement of Ebola virus by nucleic acid tests. The role of the International Standard is to harmonise this measurement. By calibrating a clinical laboratory's data against the International Standards, variability in assay results between laboratories is considerably reduced. This is important for accuracy of patient diagnosis as well as those tests being used to analyse clinical trials.

Q7

You are also involved in quality control testing of products used in childhood vaccination programmes. How have these been affected by the COVID-19 pandemic and the roll-out of COVID-19 vaccines?

"The COVID-19 vaccines, while addressing a new disease, add to the considerable specialist expertise the NIBSC has across a wide range of pathogens and vaccines."

The role of the NIBSC's independent batch release activity covers vaccines that are used in routine immunisation programmes, including those for children. All of these vaccines are still required to prevent the resurgence of controlled viral and bacterial disease, and thus the quality assessment has continued to support their supply and public health. New vaccines are made available periodically and medicines control laboratories such as the NIBSC apply their scientific and regulatory expertise to establishing appropriate quality tests to verify the quality of the product and support deployment of the new products. The COVID-19 vaccines, while addressing a new disease, add to the considerable specialist expertise the NIBSC has across a wide range of pathogens and vaccines.

Sarah Caddy

Clinical Research Fellow in Viral Immunology, Department of Medicine, University of Cambridge, UK

Q1 What initially sparked your interest in viral immunology and vaccines?

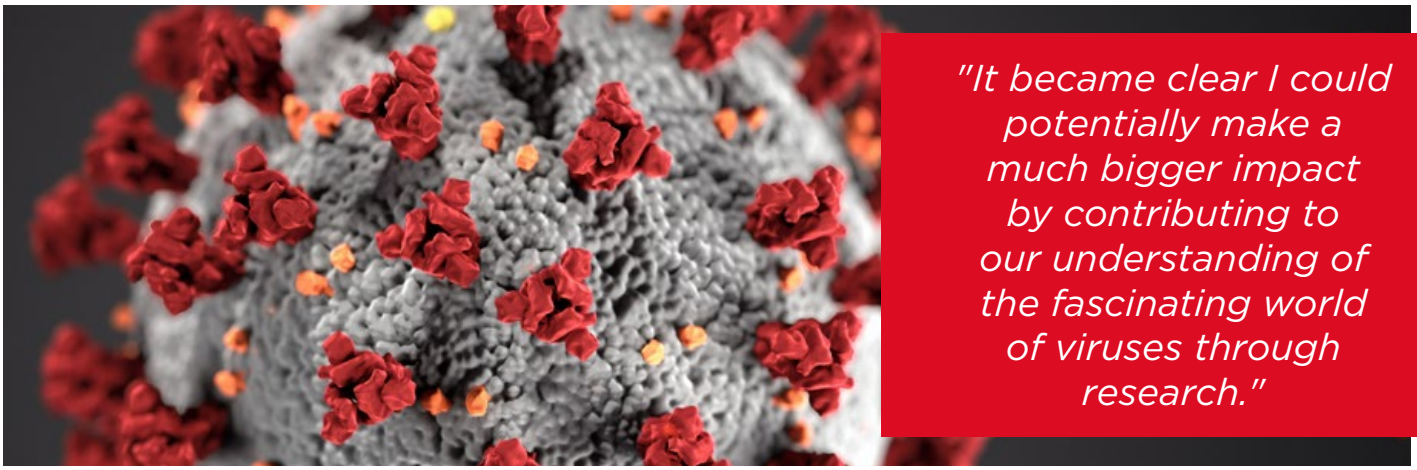
I initially trained as a veterinary surgeon, and spent several years vaccinating animals and treating animals suffering from viral diseases. However, I realised that I was only making a difference to a small number of animals by working in clinics. It became clear I could potentially make a much bigger impact by contributing to our understanding of the fascinating world of viruses through research. At the molecular level there is little to differentiate viruses of animals and humans, so my work now bridges virology and immunology relevant to all species.

Q2 How did your previous work on studying norovirus and Ebolavirus help you transition to studying severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)?

My PhD focussed on zoonotic aspects of noroviruses, and for part of this I conducted a large quantitative polymerase chain reaction-based screen for several norovirus strains. The end of my PhD coincided with the recent Ebola outbreak in West Africa, so I was then able to volunteer my molecular skills in a diagnostic and research lab in Sierra Leone, testing patient samples for Ebola by quantitative polymerase chain reaction and performing virus sequencing. When the SARS-CoV-2 pandemic reached the UK, this meant that I immediately wanted to offer my expertise in a similar capacity. I never expected the skillset gained in Sierra Leone would one day be valuable in Cambridge.

Q3 As a volunteer with the COVID-19 Genomics UK (COG-UK) Consortium, could you tell us how genomic data is being used to aid control of COVID-19?

At the start of the first lockdown in March 2020 I joined the Cambridge arm of the COG-UK consortium for 2 months. This involved performing full genome sequencing for all of the SARS-CoV-2 positive samples within the region. When these results were analysed alongside detailed epidemiological data, it was possible to identify a number of hospital-acquired cases of the virus. As we were able to generate sequencing data within days of a positive sample, this led to implementation of more effective infection control measures.



"It became clear I could potentially make a much bigger impact by contributing to our understanding of the fascinating world of viruses through research."

"My current main research focus is understanding how antibodies protect us from viruses. In particular I have been interested in revealing the role of 'non-neutralising' antibodies."

Since this time, COG-UK has been able to identify and monitor any changes in virus sequence across the country, which has proven to be of significant importance as new virus variants emerge.

Q4

There are now several known variants of SARS-CoV-2. Is there ways of preventing the virus mutating, and is there a way of predicting such mutations?

Viruses with an RNA genome inevitably mutate due to inaccuracies in genome replication. This is advantageous to the virus as, although the majority of mutations will have no effect on virus survival, a few will enable the virus to evade immune responses. Unfortunately, this means that there is no way to stop viruses mutating, although the impact will be less if there are fewer infections. Predicting mutations is possible though, and has been done via a number of ways. One method is through computational analysis and comparison to similar viruses. The other way is to grow virus in the lab and repeatedly use it to infect new cells in the presence of antibodies. This immune pressure can often induce the emergence of virus variants that can replicate despite the presence of inhibitory antibodies.

Q5

You recently co-authored a paper titled: 'Viral nucleoprotein antibodies activate TRIM21 and induce T cell immunity.' Could you summarise the key take-home messages of this article?

My current main research focus is understanding how antibodies protect us from viruses. In particular I have been interested in revealing the role of 'non-neutralising' antibodies. These are antibodies targeting internal viral antigens that don't block virus infection *in vitro*, but we know they are protective *in vivo*. Our work has shown that non-neutralising antibodies can actually enhance antigen presentation via the intracellular antibody receptor TRIM21. This therefore leads to enhanced activation of T cells, shown to be critical for survival from infection in our mouse models. Overall, this means that testing for neutralising antibodies alone may miss important correlates of protection. It also suggests that inclusion of internal viral proteins in vaccines may be valuable.

Q6

At the Cambridge Institute for Therapeutic Immunology and Infectious Diseases (CITIID), you have previously developed a project for COVID-19 testing in animals. What evidence is there on COVID-19 pet transmission?

There is good evidence that experimentally infected cats are susceptible to SARS-CoV-2, and there are a number of case reports of pet cats and a few pet dogs testing positive for the virus. However, there is no evidence that pets can transmit the virus back to humans. The story is very different for mink as there is now solid evidence based on sequencing data that mink can transmit the virus to people. But for cat and dogs, although transmission to humans remains a theoretical risk, this is deemed to be extremely low.

Q7

One of your research interests is understanding how viral vaccines work in infants and how they can be improved. What new developments in this field are on the horizon in 2021?

Vaccine efficacy in very young infants is often reduced due to the presence of maternal antibodies. This is a phenomenon that has been recognised for decades across multiple species, but the mechanisms of inhibition are still not clear. One solution to this problem is to focus on vaccinating mothers, and then relying on maternal antibodies to protect infants. This is likely to be the strategy that will be employed in 2021 for the SARS-CoV-2 vaccines. But we know maternal antibodies wane at different rates in infants, which means some infants will be unprotected whilst still very young. New vaccines for infants that can somehow overcome inhibition by maternal antibodies are a long sought-after goal.

Gross, Histopathological, and Ultrastructural Features in Patients with COVID-19: A Literature Review

EDITOR'S PICK

My choice for the Editor's Pick this issue is the highly relevant article by Hussain et al. Although rapid efforts have been made to elucidate the clinical course, prognostic markers, and complications of severe acute respiratory syndrome coronavirus-2, its pathophysiology remains poorly understood. In this fascinating and timely study, the authors provide a much-needed overview of the biopsy, gross autopsy, and other histopathological findings that have been reported in various organs in people with COVID-19. Given the current global pandemic, the importance of this literature review cannot be overstated.

Rajeshwar Reddy Kasarla

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Abstract

The coronavirus disease (COVID-19) outbreak has led to swift efforts to learn about its clinical course, prognostic markers, and complications. Consequently, there is a lot of scattered information available regarding severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) but its pathophysiology is still poorly understood. Gross and microscopic findings are very important for understanding any disease, including COVID-19. This literature review examines and summarises the biopsy, gross autopsy, and other histopathological findings that have been reported in various organs in COVID-19 patients to increase the understanding of the disease. Many histopathological findings in various organs were nonspecific, especially in the liver and

brain, while others were particular to SARS-CoV-2. Therefore, further histopathological studies and autopsies are necessary to obtain consistent and reliable findings in those with COVID-19 to fully understand the pathogenesis of the disease and the impact it has on individual organs.

INTRODUCTION

The analysis of biological samples has always played a vital part in understanding pathological processes of diseases. As the outbreak of the coronavirus disease (COVID-19) turned into a global pandemic, new information has emerged regarding various aspects of the disease. However, the current understanding about the precise nature and reaction patterns in various organs and tissues in response to this infection is lacking and poorly understood. In the large number of original studies available on the subject matter, it can be difficult to get a full picture of the effect that COVID-19 has on the body as a whole, as many studies show conflicting results. In this literature review, the authors examined and summarised the macro- and micropathological findings present in each organ system in a COVID-19 patient in already published literature.

METHODS

Until 11th July 2020, PubMed and Embase were searched for related published articles. In both electronic databases, the following search strategy was applied and these keywords (in the title or abstract) were used: "COVID 19" OR "coronavirus" AND "biopsy" OR "autopsy" OR "histopathology".

RESULTS

Lungs (n=215 cases)

A reported gross examination was available for 89 cases that tested positive for COVID-19. The macroscopic examination in all cases showed findings that ranged which patchy to diffuse areas of consolidation to severe and extensive suppurative infiltrates. A common finding was an increased lung weight in 70 cases;[1-9] they were more than one standard deviation heavier than average weight. Lungs consisting of firm parenchyma with regions of dark-coloured to reddish haemorrhagic areas, with focal demarcation and severe congestion were found in 38 cases.[3-5,7-12] Visible pulmonary thromboembolism were noted in 27 cases,[3,13-16] some of them with associated infarcts after prophylactic dose anticoagulant therapy, suggesting that pulmonary thrombi were formed despite anticoagulant therapy.[13] In some cases, visibly enlarged peritracheal and peribroncheal lymph nodes were found.[7,10,11]

Histopathological examination was available for 190 COVID-19 positive cases. The most commonly reported histopathological finding was diffuse alveolar damage, reported in all but one case[17] ranging from hyaline membrane formation, alveolar wall oedema, fibrin deposits within the alveoli, severe capillary congestion and severe syncytial cell and Type II pneumocyte hyperplasia, and desquamation to diffuse necrosis of alveolar lining cells.[2,4-6,8-13,15,18-27] One recently performed prospective cohort study reported that diffuse alveolar damage was the primary abnormality in hospitalised patients and almost every patient positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) died without medical intervention.[1] Predominantly, inflammatory CD3+ and CD4+ T cells were found near precapillary and postcapillary vessel walls along with macrophages and abundant polymorphonuclear leukocytes whereas the number of CD8+ T cells was lower in comparison in most cases.[2,5,6,10,16,17,19,28,29] CD61+ megakaryocytes were abundantly found actively producing platelets and located within pulmonary capillaries, further indicating the stimulation of the coagulation cascade.[6,5,20]

Another broad finding was pulmonary thromboembolism with fibrinous thrombi in small and large pulmonary arterioles, in some cases with haemorrhagic foci.[5,6,20-23,30] In a comparative study, the lungs presented structurally deformed capillaries, showing abrupt calibre changes such as intussusceptive pillars within the capillaries and hallmarks of endothelial cell swelling, loss of contact with basal membrane, and disruption of intercellular junctions on electron microscopy.[2] In multiple cases, ultrastructural examination

showed distended cytoplasmic vacuoles within the pneumocytes with viral cytopathic changes and intracellular SARS-CoV-2. Virions were seen extracellularly among the cilia and within the cytoplasm of respiratory epithelial cells in the upper airway.[2,6,7,10,14,18,22,27] Immunohistochemistry of viral antigens was positive in the upper airway, bronchiolar and submucosal gland epithelium, Type I and II pneumocytes, alveolar macrophages, and lung hyaline membranes.[22] Superimposed bronchopneumonia was described in 19 cases with mostly neutrophilic infiltrate.[4,19,20]

Cardiovascular system (n=67 cases)

Cardiovascular gross findings were reported in 19 autopsy cases that tested positive for COVID-19. The most commonly reported gross findings in seven cases were large vessel occlusions characterised by intimal and medial thickening with luminal narrowing.[31-33] Other reported gross findings in two cases included thin myocardial trabecula, streaking of right atrial wall myocardial tissue,[7] and mottling parenchyma with a soft and rubbery texture.[6] The most significant gross findings generally seen were cardiomegaly and right ventricular dilatation, which were attributed to pre-existing underlying diseases such as hypertension, diabetes, and obesity. No significant gross findings were observed in the remaining 10 cases.

Histopathological autopsies findings were available for 59 cases of COVID-19 positive patients. Unlike in other similar viral infections that caused viral myocarditis, there were no reported cases of COVID-19 myocarditis. The most commonly reported histopathological finding was myocyte necrosis, observed in almost all the cases except for one case.[7] Changes to the myocardium included irregularity in shape with a darkened cytoplasm, though these changes were not sufficient for interpretation as acute myocardial injury in four cases;[24] mild myxoid oedema; mild myocyte hypertrophy; focal nuclear pyknosis, in one case;[7] and moderately enlarged cardiomyocyte with hyperchromatic nuclei and vacuolar degenerative change in three cases.[31] The presence of diffuse lymphocytic endothelitis and apoptotic bodies were shown in 34 cases.[32-35]

A study proposed that SARS-CoV-2 might not directly impair the heart, because they observed scarce interstitial mononuclear inflammatory infiltrates in the myocardial tissue without substantial cardiac muscle tissue damage in their patient with COVID-19.[36] There was also evidence of direct viral infection of endothelial cells, endothelitis with diffuse endothelial inflammation, and micro- and macrovascular thrombosis, both in the venous and arterial circulations, in 26 cases.[34]

In conclusion, cardiomegaly was most likely a result of pre-existing heart disease. Although histopathological cardiac findings were notable for absence of lymphocytic myocarditis, myocyte necrosis was the most commonly reported finding.

Gastrointestinal (n=24)

The most common gross finding was multiple and diffuse punctate haemorrhages in the stomach (n=3) and duodenal mucosa (n=1).[37,38] Other gross findings (n=2) included increased liver volume and an enlarged gallbladder.[37,38]

In all of the 24 cases, there was mild-to-moderate hepatic lobular or portal lymphocytic infiltration.[18,19,24,38,39] Four cases showed centrilobular sinusoidal dilatation.[24] Minimal-to-mild micro- and macrovesicular steatosis was seen in ten cases.[18,19,38] Patchy and multifocal hepatic necrosis were seen in 13 cases.[19,24,38] These findings were mostly nonspecific and can be attributed to direct viral-induced cellular injuries and potential hepatotoxicity from therapeutic drugs, or be representative of pre-existing chronic liver disease that was exacerbated during COVID-19, and COVID-19-related hyperinflammatory reactions.[40] SARS-CoV-2 particles without membrane-bound vesicles in hepatocyte cytoplasm were also identified.[38]

Central nervous system (n=79 cases)

Clearly reported gross examination was available for 41 cases that tested positive for COVID-19. The macroscopic examination showed findings that ranged from mild swelling and cerebral oedema in three cases,[4,41,42] to disseminated haemorrhagic lesions throughout the cerebral white matter (size ranging from 1 mm to 1 cm) and scattered punctate subarachnoid hemorrhages in two cases.[41,43] Hydrocephalus internus was present in two cases.[4] No abnormalities were seen in 23 cases

apart from those which were already associated with the patients' prior comorbidities such as atherosclerosis and Parkinson's disease (n=16).[4]

Histopathological examination was available for 65 COVID-19 positive cases. Here, 33 cases showed no microscopic abnormalities; one case mimicked glioma on imaging but on histopathological examination was negative for malignancy and positive for encephalitis.[44] Reactive gliosis, neuronal satellitosis, perivascular haemorrhage,[45] and disseminated haemorrhagic cerebral white matter lesions[41] were found in eight, five, one, and one case, respectively. Other neuropathological findings included characteristics resembling vascular and demyelinating lesions. For example, the scattered necrotic neurons of acute hypoxic injury with red degeneration and oedema in the cerebrum (cortex), cerebellum (Purkinje cell layer), and hippocampus (CA1) were observed in 19 cases.[41,46] Infarcts in brainstem, deep grey nuclei, or spinal cord were absent. In one case, the subcortical white matter had macrophage clusters with axonal injury and a perivascular acute disseminated encephalomyelitis-like appearance.[41] No thrombi, vasculitis, or abnormalities of the olfactory bulb were observed in any cases.[39,41] Focal leptomeningeal inflammation was detected in one brain specimen[46] while no inflammation was present in the leptomeninges of another case.[41] Perivascular lymphocytic clusters were detected in three brain specimens.[41,46]

Kidney (n= 43)

The histopathological effects of COVID-19 in kidney tissues were reviewed for 43 cases. Here, 32 cases reported severe acute tubular necrosis, showing diffuse proximal tubular injury with the loss of brush border, vacuolar degeneration, mild fibrosis in the interstitium, and lymphocyte infiltration on microscopy while severe glomerular injury was absent.[47-49] The vacuoles seen on light microscopy correlated with double-membrane vesicles containing virion particles by electron microscopy.[49] Infrequent haemosiderin granules, pigmented casts, and lumen-obstructing red blood cell aggregates were also observed. Vasculitis, interstitial inflammation, or haemorrhage were absent.[48] Electron microscopy demonstrated virus-like particles are in the tubular epithelium and podocytes.[48] The virus was not only directly cytotoxic, but also had the ability to initiate CD68+ macrophage and complement C5b-9 deposition to mediate tubular pathogenesis.[47,50]

In a separate study that evaluated 10 patients with proteinuria and COVID-19, all renal biopsies showed renal tubular acidosis without evidence of the virus in the biopsied tissue.[51]

Skin (n=111)

The clinical presentation of skin in COVID-19 patients showed findings including urticarial, vesicular, and petechial or purpuric eruptions; erythema multiforme; vascular complications such as acro-ischaemia, livedo, necrosis, or gangrene and chilblain; and maculopapular eruptions including morbilliform rash, plaques, and pityriasis rosea.[52]

Histopathological examination was available for 30 COVID-19 positive cases. The most common finding, present in 18 cases, was significant degree of perivascular infiltration of the superficial dermis by lymphocytes, eosinophils, and neutrophils in vasculitic pattern with accompanying papillary oedema and extravasation of red blood cells.[44,53-58] Thrombogenic vasculopathy, found in seven cases, was associated with widespread epidermal or papillary dermal or dermohypodermal and adnexal necrosis. Adnexal necrosis spared ducts, but was present in the secretory portion of sweat coil.[54,56,58] Other epidermal findings included spongiosis, basal cell vacuolation, suprabasal acantholytic ballooning keratinocytes, parakeratosis, and dyskeratotic.[56,57] Superficial and deep dermal lichenoid infiltrate was also found in one case of chilblains.[53] Immunohistochemistry showed extensive C5b-9 deposition within the vessels of the dermis.[54]

Testes (n=13)

Histopathological examination was available for 13 COVID-19 positive cases. Out of these, 11 cases showed swollen, vacuolated Sertoli cells. Intratubular cell detachment from basement membranes of the seminiferous tubules was noted. No inflammatory cells were found within the seminiferous tubules while the interstitium showed oedema and infiltration of T lymphocytes and histiocytes.

Table 1: Pulmonary findings in patients with coronavirus disease (COVID-19).

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
Lung	Xu et al.,[18] 2020	1	China	50	Male	Lung injury, ARDS
	Grimes et al.,[14] 2020	2	USA	Middle-age	Male	HTN, asthma, HIV compliant with antiretroviral therapy
	Ackerman et al.,[2] 2020	7	USA	Mean(±SD): 68(±9.2), and 80(±11.5)	Female: 2; male: 5	NA
	Aguiar et al.,[5] 2020	1	Switzerland	31	Female	Morbid obesity
	Duarte-Neto et al.,[45] 2020	10	Brazil	NA	NA	NA
	Beigmohammadi et al.,[19] 2020	7	Iran	Mean (range): 67.85 (46–84)	Female: 2; male: 5	HTN (57.0%), RA (14.2%), none (28.5%)
	Brown et al.,[17] 2020	1	USA	NA	NA	NA
	Deshpande, et al.,[13] 2020	11	USA	NA	NA	HTN, DM, obesity, COPD, CAD, cancer, cerebrovascular disease (4 patients), pulmonary embolism (1 patient)
	Dolhnikoff et al.,[20] 2020	10	Brazil	Mean (range): 67.8 (33–83)	Female: 5; male: 5	HTN, DM, IHD, COPD (7 patients)
	Fox et al.,[6] 2020	10	USA	Range: 44–78	NA	HTN, DM, obesity, immunosuppressed (1 patient)
	Jain et al.,[21] 2020	NA	NA	NA	NA	NA
	Konopka et al.,[1] 2020	8	USA	Average (range): 53 (22–80); 49 (33–63)	Female: 6; male: 2	Idiopathic bronchiectasis and pneumonia, suffered acute intraparenchymal brain haemorrhage (1 patient)
	Lax et al.,[3] 2020	11	Austria	Mean/median (range): 81.5/80.5 (75–91)	Female: 3; male: 8	HTN (81%), DM (45%), cerebrovascular disease (36%), dementia (36%)
	Li et al.,[40] 2020	NA	China	NA	NA	NA
	Martines et al.,[22] 2020	8	USA	Median: 73.5 (two <65)	NA	HTN (75%), CKD (75%), cardiovascular disease (75%), obesity (62.5%), DM (50%)
	Menter et al.,[4] 2020	21	Switzerland	Mean (range): 76 (53–96)	Female: 4; male: 17	HTN (100%), cardiovascular disease (71%), smoker (38%), pre-obesity/obesity, DM (33%), chronic neurological condition (23.8%), COPD (14.2%), malignancy (14.2%), CLD (9.5%), CKD (19%), acquired immunosuppression (4.7%)
	Pernazza et al.,[23] 2020	1	Italy	61	Male	Lung adenocarcinoma

Table 1 continued.

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
	Popa et al.,[10] 2020	1	Romania	88	Male	HTN, DM, with permanent electrical cardio-stimulation, history of TIA, immobilised in bed
	Scendoni et al.,[15] 2020	2	Italy	Female: 62; male: 44	Female: 1; male: 1	Male: polymyositis Female: HTN, autoimmune hypothyroidism, DM
	Schaefer et al.,[16] 2020	7	USA	Average: 62.4	Female: 2; male: 5	SLE, RA, pulmonary fibrosis, CKD, interstitial lung disease, MGUS, CAD, HTN, DM, cardiomegaly, atherosclerosis, dementia, status post-leg amputation, COPD, NASH
	Shao et al.,[29] 2020	1	China	65	Male	None
	Suess et al.,[11] 2020	1	Switzerland	59	Male	HTN, DM
	Tian et al.,[12] 2020	2	China	Female: 84; male: 73	Female: 1; male: 1	Male: HTN, DM, adenocarcinoma
	Tian et al.,[24] 2020	2	China	NA	NA	NA
	von der Thüsen, van der Eerden[25] 2020	41	The Netherlands	NA	NA	NA
	Wang et al.,[26] 2020	2	China	Female: 53; male: 62	Female: 1; male: 1	Female: DM and HTN
	Yan et al.,[7] 2020	1	USA	44	Male	Obesity
	Zeng et al.,[27] 2020	1	China	55	Female	None
	Zhang et al.,[28] 2020	1	China	72	Male	DM and HTN
	Conde et al.,[8] 2020	1	Spain	69	Male	None
	The COVID-19 Autopsy,[9] 2020	1	Spain	54	Male	HTN, gout, migraine, OSA
	Schaller et al.,[39] 2020	12	Germany	Median: 79	Female: 3; male: 7	HTN (41%), DM (16%), COPD (16%), malignancy (16%), obesity (16%)
	Tian S et al.,[24] 2020	4	China	Median: 73	Female: 1; male: 3	DM and HTN

ARDS: acute respiratory distress syndrome; CAD: coronary artery disease; CKD: chronic kidney disease; CLD: chronic liver disease; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; HTN: hypertension; IHD: ischaemic heart disease; MGUS: monoclonal gammopathy of undetermined significance; NA: not available; NASH: non-alcoholic steatohepatitis; OSA: obstructive sleep apnoea; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; TIA: transient ischaemic attack.

Table 2: Cardiovascular findings in patients with coronavirus disease (COVID-19).

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
CVS	Becker et al.,[32] 2020	4	USA	Mean: 50	Male	NA
	Buja et al.,[31] 2020	4	USA	Range: 44–76	Female: 1; male: 3	Obesity, DM, CKD
	Centurión et al.,[36] 2020	15	Paraguay	NA	NA	NA
	Craver et al.,[35] 2020	1	USA	17	Male	NA
	Fox et al.,[6] 2020	10	USA	Range: 44–78	NA	HTN, obesity, DM
	Pons et al.,[34] 2020	26	France	Mean: 65	NA	NA
	Tian et al.,[24] 2020	4	China	Range: 59–81	Female: 1; male: 3	Pneumonia
	Varga et al.,[33] 2020	3	Switzerland	Range: 58–71	Female: 1; male: 2	HTN, renal transplant patient

CKD: chronic kidney disease; CVS: cardiovascular system; DM: diabetes mellitus; HTN: hypertension; NA: not available.

Table 3: Findings in other organ systems in patients with coronavirus disease (COVID-19) (gastrointestinal tract, central nervous system, renal, skin, testes).

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
GIT	Wang et al.,[38] 2020	156	China	Mean: 51	Male: 82	DM, HTN, digestive system disease, endocrine system disease, nervous system disease, chronic respiratory disease
	Schaller et al.,[39] 2020	10	Germany	Mean (range): 79 (64–90)	Male: 7	CAD, COPD, CKD, DM, morbid obesity, endocrine system disease, CML, adenocarcinoma of the lung
	Beigmohammadi et al.,[19] 2020	7	Iran	Mean (range): 67.9 (46–84)	Male: 5	DM, HTN, CAD, digestive system disease, RA
	Xu et al.,[27] 2020	1	China	50	Male: 1	NA
	Adachi et al.,[37] 2020	1	Japan	84	Female: 1	NA
	Tian et al.,[24] 2020	4	China	Mean (range): 73 (59–81)	Male: 3	CLL, CLD, HTN, DM, renal transplantation
CNS	Duarte-Neto et al.,[20] 2020	9	Brazil	Median (range): 69 (33–83)	NA	NA

Table 3 continued.

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
	Menter et al.,[4] 2020	18	Switzerland	Median (range): 76 (53–96)	Female: 4; male: 17	HTN, CAD, DM, ESRD, obesity, chronic neurological condition, CLD
	Reichard et al.,[41] 2020	1	USA	71	Male: 1	CAD
	Liu et al.,[42] 2020	1	NA	NA	NA	NA
	Bradley et al.,[43] 2020	14	USA	Median (range): 74 (42–84)	Female: 8; male: 6	HTN, CKD, OSA, DM, obesity
	Efe et al.,[44] 2020	1	NA	35	Female: 1	None
	Solomon et al.,[46] 2020	18	NA	Median (interquartile range): 62 (53–75)	Female: 4; male: 14	DM (12 patients), HTN (11), CAD (5), hyperlipidaemia (5), CKD (4), prior stroke (4), dementia (4), treated anaplastic astrocytoma (1)
	Schaller et al.,[39] 2020	10	Germany	Median (range): 79 (64–90)	Male: 7	Leukaemia, HTN, hypothyroidism, COPD, CKD, cardiomyopathy, obesity, DM, atrial fibrillation, CAD
Kidney	Su et al.,[48] 2020	26	China	69	Female: 7; male: 19	Renal failure, multiple organ dysfunction, clinical signs of kidney injury (nine patients)
	Diao B et al.,[47] 2020	6	China	Range: 21–92	NA	HTN (three patients)
	Farkash EA et al.,[49] 2020	1	China	53	Male: 1	Aortic dissection repair complicated by renal failure, obesity, hyperlipidaemia
	Sharma P et al.,[51] 2020	10	China	Mean: 66	Female: 5; male: 5	DM, HTN
Skin	Duarte-Neto et al.,[20] 2020	12	Brazil	Median (range): 69 (33–83)	NA	NA
	Kolivras et al.,[53] 2020	1	NA	23	Male: 1	Psoriasis
	Magro et al.,[54] 2020	5	NA	Range: 32–73	Female: 2; male: 3	Females: no comorbidities Males: CAD, DM, heart failure, ESRD, obesity
	Diaz-Guimaraens et al.,[55] 2020	1	Spain	48	Male: 1	HTN
	Gianotti et al.,[56] 2020	3	Italy	Range: 57–89	Female: 2; male: 1	NA
	Ahouach et al.,[57] 2020	1	NA	57	Female: 1	NA
	Llamas-Velasco et al.,[58] 2020	1	NA	61	Male: 1	DM

Table 3 continued.

Organ	Study	Number of cases	Country of origin	Age (years)	Sex	Comorbidities
Testes	Duarte-Neto et al.,[20] 2020	2	Brazil	Median (range): 69 (33–83)	Male: 1	NA
	Yang et al.,[59] 2020	11	China	Mean (range): 65 (42–87)	Male: 1	HTN, CKD, CAD, neoplasia (two patients had no comorbidities)

CAD: coronary artery disease; CKD: chronic kidney disease; CLD: chronic liver disease; CLL: chronic lymphocytic leukaemia; CML: chronic myelogenous leukaemia; CNS: central nervous system; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; ESRD: end-stage renal disease; GIT: gastrointestinal tract; HTN: hypertension; NA: not available; OSA: obstructive sleep apnea; RA: rheumatoid arthritis.

Out of the same 11 cases, 18.2%, 45.5%, and 36.4% of cases showed <10%, 10–50%, and >50% injury to seminiferous tubules, respectively, while mild to no tubular injury was present in five cases of COVID-19 negative controls. The Leydig cell count was also significantly lower than in the control group. Normal spermatogenesis was observed in all cases as ACE-2 was not expressed in spermatogonia but widely present in Sertoli and Leydig cells.[59] The rest of the two cases showed orchitis.[45]

Table 1, 2 and 3 show the patient demographics such as sex, age, comorbidities, and country the study was conducted in.

LIMITATIONS AND RECOMMENDATIONS

The data or cases available for review were very limited as many hospitals discontinued autopsies on COVID-19 cases to limit the spread of the virus. Further, larger studies are needed to assess the effect of COVID-19 in different organs, especially on the central nervous system, kidneys, and the gastrointestinal tract. The authors also recommend that more studies are conducted in areas with higher prevalence of comorbidities, such as viral hepatitis or tuberculosis, so that the effects of specific pre-existing conditions on the outcome of the virus can be determined. Studies comparing the effects of COVID-19 on various organs in healthy patients versus in those with pre-existing conditions will also be helpful.

CONCLUSION

In conclusion, the many findings in multiple organs could not be definitively attributed to SARS-CoV-2 as many patients had underlying pre-existing conditions; therefore, further case-control type studies are required to credit the histopathological findings to SARS-CoV-2.

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The PPE Pandemic: Sex-Related Discrepancies of N95 Mask Fit

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Abstract

During the COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2, healthcare professionals across the world have been at high risk of transmission because of their direct contact with infected patients. In October 2020, the International Council of Nurses (ICN) confirmed that 1,500 nurses had died from COVID-19 in 44 countries and estimated that healthcare worker COVID-19 fatalities worldwide could be more than 20,000. To ensure protection of healthcare personnel, properly fitting personal protective equipment (PPE) must be worn. In a 'one size fits all' world, the differences between the fit of PPE for men and women can have devastating consequences. An N95 respirator mask is a component of PPE outlined by the Centers for Disease Control and Prevention (CDC) for protection against COVID-19; however, N95 masks do not offer protection if they do not fit properly. Fit testing is performed to ensure an adequate seal of the mask on the wearer. A single-institution retrospective review was performed on fit testing results for male versus female wearers in an attempt to elucidate a difference in failure rates. Females failed at a significantly higher rate than their male counterparts (6.67% female, 2.72% male; p=~0.22 [with the continuity correction; ~0.14 without it]), and the reason reported was often due to being 'small-boned' (p<0.0001). Sex-related differences in proper PPE fit are not new; however, the COVID-19 pandemic has made the situation more acute, and sex-specific N95 mask designs must be developed quickly, as the pandemic shows little signs of abating.

INTRODUCTION

Novel Coronavirus Disease 2019

In December 2019, cases of pneumonia spiked in Wuhan, China. The cause of the acute respiratory illness was identified as a novel β-coronavirus. This coronavirus was named the 2019-novel coronavirus on 12 January 2020 by the World Health Organization (WHO). On 11th February 2020, the WHO officially named the respiratory disease as COVID-19 and the Coronavirus Study Group (CSG) of the International Committee proposed to name the new coronavirus, severe

acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1]

As cases increased, human-to-human transmission via respiratory droplets was recognised.[2]

Transmission to healthcare workers caring for infected patients was described on 20 January 2020, and the need for personal protective equipment (PPE) for healthcare professionals became imperative. The use of PPE such as the N95 masks became a standard need. Healthcare workers are at increased risk of infection from COVID-19 because of their close proximity with infected patients. Infection rates of healthcare workers have been reported as high as 29% in China, 20% in Italy, 6% in the Netherlands, and 3% in the United States.[3,4] In particular, healthcare workers from Black, Asian, and minority ethnic backgrounds are at increased risk of infection.[5] As the pandemic continues, access to properly fitting PPE for healthcare workers is an important concern.

Personal Protective Equipment

The COVID-19 pandemic has caused a need for PPE like the world has never encountered before. The use of PPE reduces but does not fully eliminate the risk of transmission of highly infective agents.[6] According to the Centers for Disease Control and Prevention (CDC), proper PPE for COVID-19 includes a face shield or goggles, a N95 or higher respirator, one pair of clean non-sterile gloves, and an isolation gown.[7] The requirement for the use of N95 respirator masks is based on the current understanding of SARS-CoV-2 and related respiratory viruses.[8] Current data suggests that likely routes of transmission of SARS-CoV-2 include close-range aerosol transmission by droplet and inhalation, and contact followed by self-inoculation via delivery to the eyes, nose, or mouth.[8] N95 and higher-level respirators provide barrier and respiratory protection because of their tight fit and filtration characteristics; however, this fit must be assessed for effectiveness with a respirator fit test.[8]

Particulate respirators, such as N95 masks, have a non-woven fibrous filter media that captures particles. N95 masks have been shown to filter 99.8% of particles with a diameter of approximately 0.1 µm. SARS-CoV-2 has a diameter of 0.1 µm, making N95 masks capable of filtering the viral particle.[9,10] In addition, N95 masks offer protection from the transmission of the virus by filtering respiratory droplets produced via coughing and sneezing.

Respirators are available in different models and sizes. Respirator fit testing requires selection of the most appropriate model. It includes the proper process of donning and requires at least 5 minutes of wear to assess comfort and effectiveness.[11] The United States Department of Labor (DOL) reports the following criteria must be assessed to help determine the adequacy of respirator fit: chin properly placed, adequate strap tension, fit across nose bridge, respirator of proper size to space distance from nose to chin, tendency of respirator to slip, and self-observation in mirror to evaluate fit and position.[11] The test subject must then conduct a user seal test and undergo test exercises to determine if the fit is adequate. If the respirator doesn't fit properly, contaminated air can leak into the facepiece and potentially cause the wearer to breathe in hazardous substances.[12]

Proper respirator fit is vital for protection of the wearer. Previous reports have stated that facial hair, such as a beard, could prevent the mask from properly fitting; however, data regarding the differences in fit between male and female wearers is lacking.[6,11] Initial fit pass rates for filtering facepiece respirators vary widely, with lower pass rates found in women and Asians.[13] Higher initial fit pass rates were found in Caucasians (90%) compared with Asians (84%), and particularly low initial first pass rates were reported in Asian females, with a reported mean of 60%.[14]

Females, regardless of height and weight, have smaller bone structures than males, and most PPE has been designed for the male body,[15] yet, according to the United States Census Bureau, in 2019, females held 76% of all healthcare jobs.[16,17] Improper fit of PPE can exacerbate the exposure to the virus by female healthcare and essential workers. A report published by the International Council of Nurses (ICN) on 3rd June 2020 indicates that over 230,000 healthcare workers have contracted COVID-19, and more than 600 nurses have died from the virus.[18] In another study investigating the characteristics

Figure 1: 3M™ (Saint Paul, Minnesota, USA) Health Care Particulate Respirator and Surgical Mask 1860, N95 120 EA/ Case.

and related factors of COVID-19 infection in healthcare workers, it was found that 45 (29.80%) infected healthcare workers were male and 106 (70.20%) were female.[19] In European Union (EU) countries, a higher proportion of healthcare workers diagnosed with COVID-19 infection were female.[20]

Additionally, in Spain, 72% of infected healthcare workers were female (5,265) and in Italy 66% of infected healthcare workers were female (10,657). Similar trends were found in the USA, with the CDC reporting 73% of infected healthcare workers were female.[20] Therefore, it is important to ensure proper N95 fit for all healthcare workers, including females, since they make up the majority of the healthcare workforce.

MATERIALS AND METHODS

A retrospective review was performed on individuals who underwent respirator fit testing from December 2019 to June 2020 at a single institution. All fit testing included was performed by the staff at the health services centre on campus. The test uses an instrument to measure leakage around the face seal, resulting in a number called the 'fit factor'. The centre used Occupational Safety and Health (OSHA) test protocols.[11] Quantitative data for fit testing was collected via the PortaCount Respirator FIT tester 8038 to determine the adequacy of the seal. Two different sized masks were available for fit testing (Model 3M1860 [3M; Saint Paul, Minnesota, USA), Regular/Small) (Figure 1). Fit test results were divided into two groups, male or female, and were evaluated for failure rates. Presented data is pooled from testing of both sizes. If the first mask size tested failed, the subsequent size was tested. If both sizes failed testing, then a failure and qualitative reason for failure was recorded. Reasons for fit test failures were reported as: large boned, small boned, facial asymmetry, facial hair, and/or no reason reported (Figure 2). Exclusion criterion included failure due to facial hair. A chi-squared analysis was performed on the data to determine statistical significance.

RESULTS

A total of 336 tests were performed during this time period. There were 34 failures for all males tested versus 13 failures for females. Of the 34 male failures, 31 were due to having facial hair, and were therefore excluded from the analysis.

The data analysed included 305 total respirator fit test results: 110 male and 195 female. Out of

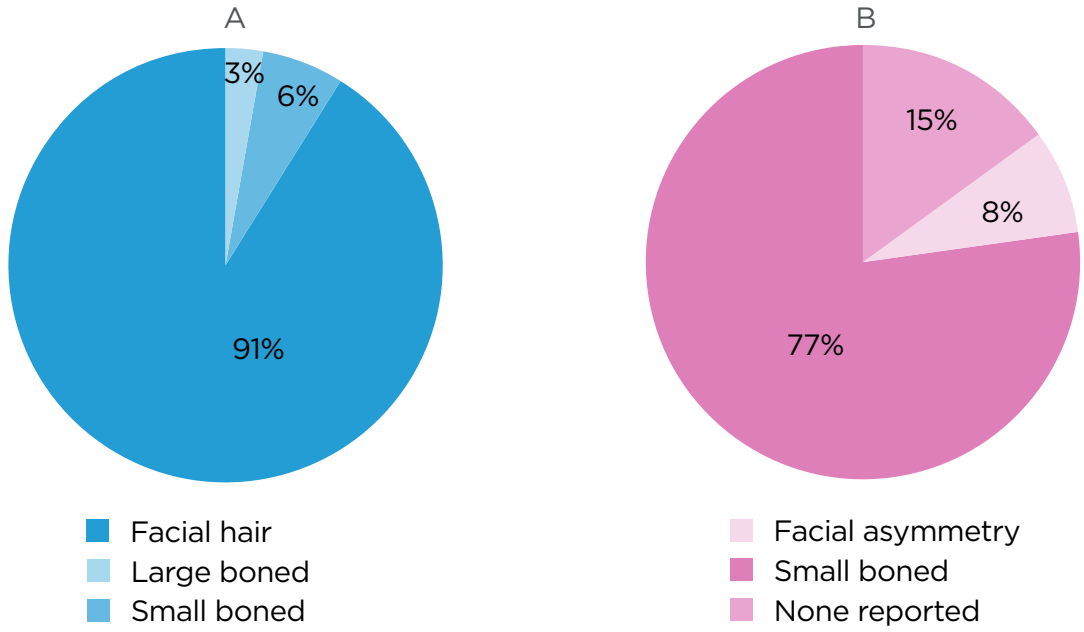

Figure 2: Sex-specific reasons for fit testing failures among A) male and B) female test subjects.

Reasons reported for respirator fit testing failures for both male and female test subjects, with their respective p-values. Failure reasons included facial hair, large boned, asymmetry, small boned, and no reason reported. Male failure total: 34, with three due to non-facial hair reasons (large boned: n=1; small boned: n=2). Female failure: 13 (facial asymmetry: n=1; small boned: n=10; none reported: n=2). Females failed significantly more due to being small boned when compared to male reasons for failure (p<0.00001).

the 110 males, 107 passed fit testing and three failed, with a failure rate of 2.72%. Out of the 195 females, 182 passed fit testing and 13 failed, with a failure rate of 6.67% (Table 1). Statistical analysis comparing expected versus observed outcomes of fit testing in males versus females indicated that females fail at higher rates than males (p=~0.22 [with the continuity correction; ~0.14 without it]) (Table 1).

DISCUSSION

Improper sizing of PPE is not a new problem; however, the COVID-19 pandemic has made the situation more acute. This study was designed to determine statistical significance of the fit of N95 respirator masks, an important piece of PPE for COVID-19 protection, comparing male and female wearers.

Proper fit of PPE for healthcare workers is crucial for protection during the COVID-19

Table 1: Total of 305 fit tests performed and analysed: 110 male and 195 female. Failure rates for male and female test subjects were 2.72% and 6.67%, respectively (p=~0.22 [with the continuity correction; ~0.14 without it]).

	Male (facial hair included)	Male (facial hair excluded)	Female
Pass	141	107	182
Fail	34	3	13
Failure Rates	31.80%	2.72%	6.67%
p-value	NA	p=~0.22 (with the continuity correction; ~0.14 without it)	

NA: not applicable.

pandemic, not just for the wearer but also for patients. Ineffectiveness of PPE may contribute to nosocomial transmission of COVID-19.[6,21] Compared with the general population, healthcare workers have an increased risk of testing positive for COVID-19, even after adjusting for testing eligibility.[22] Compared with non-healthcare workers, healthcare workers were younger (43 versus 53 years; p<0.001) and more likely female (118/193 [61%] versus 1,211/2,649 [46%]; p<0.001).[23]

One cluster randomised study attempted to compare non-fitted N95 masks versus fitted N95 masks and found that all outcomes (clinical respiratory illness, influenza-like illness, laboratory confirmed respiratory viral infection, and influenza) were consistently lower in the fitted N95 group.[24] In the present study, male wearers failed fit testing at a significantly lower rate than females (2.72% male, 6.67% female; p=~0.22 [with the continuity correction; ~0.14 without it]) (Table 1). Currently, N95 masks and other PPE equipment are made in 'one size fits all' and do not consider the differences that facial structure due to sex can have on the fit.

Reasons for male fit testing failure due to facial hair were excluded because numerous studies have shown that beard growth at points where the face and respirator come in contact prevents a good seal.[25,26] In fact, a study assessing the impact of facial hair on quantitative respirator fit in male healthcare workers showed that no full-bearded healthcare workers achieved an adequate fit, and adequate fit decreased significantly with increasing facial hair (p<0.01 for trend).[26] Therefore, if the wearer chooses to maintain facial hair, the OSHA recommends the use of respirators that do not rely on a tight facepiece seal between the respirator inlet covering and the skin (i.e., loose-fitting helmets or hoods).[25] However, as previously mentioned, facial hair is a choice, while face shape is genetically determined.

Sex-related differences in PPE fit are widespread across many different fields, not just healthcare. In 2016, poor access to appropriately fitting PPE was highlighted in a study of female construction workers. Female labourers, carpenters, and ironworkers were enrolled in semi-structured focus groups in New York City, USA. The majority reported fit problems for many types of PPE (e.g., gloves, harnesses, safety vest, work boots, and outerwear), noting that the equipment provided was too large.[27] The authors concluded that female construction workers have difficulty accessing properly fitting PPE that is designed for women.[27] A UK survey performed in May 2016 by the trade union Prospect, Women in Science and Engineering, the Trades Union Congress, and the Institution of Mechanical Engineers found that ill-fitting PPE was common but often accepted as 'part of the job'.[15]

Many responses from PPE fit surveys indicate that improperly fitting PPE hinder female's ability to do their work.[15,27,28] The challenge at hand is that PPE has been developed to fit a male frame, with smaller sizing being provided for females. However, females are not just smaller versions of men. A comparison of male and female body sizes and proportions performed in 1977 on military personnel indicated that even females of equal height and weight to their male counterparts do not have the same body proportions.[29] Unsurprisingly, facial dimensions vary with both sex and ethnic groups. A study focusing on the impact of race and ethnicity upon the development of PPE in the USA workforce showed that sex and race/ethnicity significantly differ.[30] African Americans have statistically shorter, wider, and shallower noses than Caucasians.[30] While Hispanic workers have 14 facial features that are larger than Caucasians, their nose protrusion, height, and head length are significantly shorter.[30] The final ethnic group analysed was composed of primarily Asian subjects that had statistically different dimensions from Caucasians for 16 of the anthropometric values analysed.[30] In addition, sex also significantly contributed to the size of facial features.[30,31] Even though two N95 mask sizes were offered at this institution, regular and small, females still failed for being small-boned at a significant rate (p<0.00001) due to these sex-related differences in facial dimensions (Figure 2).

A recent study also found that racial and ethnic differences throughout the pandemic

have contributed to the patterns of infection among healthcare workers.[5] Noting that healthcare workers that were Black, Asian, or Hispanic were over 3-fold more likely to contract COVID-19 compared to Caucasian healthcare workers; it is postulated that this increased incidence among underrepresented minority groups may be due to improper PPE fit. However, data is limited on this subject and this is perhaps another field for further study and another consideration in the push to redesign PPE. Facial differences by sex and gender, as well as racial and ethnic variation, lead to poorly fitting masks and may be a contributing factor to this disparity.

The Personal Protective Equipment Regulations 2002 and the Personal Protective Equipment at Work Regulations 1992 (as amended) place a legal requirement on employers to provide PPE to workers if it is needed to protect them from any workplace hazards to their safety or health.[28] Employers must ensure that the PPE is suitable for the purpose, causing significant concerns for appropriate fitting N95 masks for women health care workers.[28] The unisex approach to PPE negatively affects both the work and safety of female wearers. PPE is intended to provide protection, yet currently may be ineffective for more than 75% of the healthcare workforce. The results of this study indicate that there is a disparity in the fit of N95 masks for female wearers. Further sex-specific studies should be performed with larger sample sizes, including various ethnicities, and other proponents of PPE to ensure both statistical and clinical significance.

CONCLUSION

The COVID-19 pandemic has caused a PPE pandemic. With over 49.7 million cases worldwide and over 1.27 million deaths as of November 2020, the pandemic shows little signs of natural abatement.[32] Now, more than ever, it is important for healthcare workers to have properly fitting PPE to ensure their health and safety and the safety of those around them. In many countries, N95 respirator masks, as outlined by the CDC, are part of PPE for protection against COVID-19, but these same essential workers are faced with wearing masks that do not properly fit, leaving them at increased risk for infection. N95 masks were not designed for female facial dimensions and, because of inadequate fit, female healthcare workers are not properly protected. Developing a sex-specific approach to designing N95 masks is vital as it would protect both healthcare workers and the patients they serve.

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Impact of the COVID-19 Pandemic on Trauma and Orthopaedic Service in the Republic of Ireland

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Abstract

Background: The novel coronavirus disease-2019 (COVID-19) has been identified as the cause of a rapidly spreading respiratory illness that is thought to have originated from Wuhan, China in early December 2019. Since then, the free movement of people has decreased, which has thus reduced the number of trauma-related casualties. The Irish governments initiated strict social distancing measures in response to the COVID-19 pandemic in late March 2020. It remains challenging to quantify the impact this had on reducing the spread of the virus. The viral outbreak has led to significant changes in the lifestyle of Irish citizens. The aim of this study was to examine the impact of the pandemic on activity, related to emergencies in trauma and orthopaedics departments.

Methods: Patients admitted to the Trauma and Orthopaedic Department at Cork University Hospital (CUH), Cork, Ireland and South Infirmary Victoria University Hospital (SIVUH), Cork, Ireland between the 1st of March and the 15th of April 2020 were documented and compared to the patient admissions from the same time period in 2019.

Results: The total numbers of lower limb trauma cases dropped by 20.44% from 137 (females: 87; males: 50) to 109 (females: 74; male: 35), (p=0.72). Of note, there was a 30.00% reduction in the number of male cases (from 50 to 35; p<0.05), in comparison to the 14.94% reduction observed in the female population (from 87 to 74; p<0.05). The total number of upper limb trauma cases increased by 0.14% from 90 (female: 44; men: 46) to 94 (female: 54; male: 40; p=0.14). The increase reported was only applicable to the female population; the number of male cases of upper limb trauma slightly decreased from 46 to 40 (p<0.005).

Conclusion: The COVID-19 crisis has led to a decrease in the total numbers of lower limb trauma surgeries but an increase in the number of upper limb fractures. The main reduction was amongst the male patients, while the number of female cases increased.

INTRODUCTION

The COVID-19 pandemic is a catastrophic global healthcare crisis that is believed to have originated from Wuhan, China in late 2019[1,2] and has since spread at an exponential rate worldwide.[3] Healthcare systems have been stretched physically, financially, and mentally in an effort to curtail this crisis, with damage-limitation at the forefront

of these measures. The COVID-19 pandemic was characterised by different responses from authorities around the world. Some initiated early social distancing measures and mandatory shutdown of all nonessential outdoor activities, while others depended on thorough test and trace strategies.[4] Amongst this heterogeneity, it is difficult to quantify the effect of the singular actions and what may have occurred in the absence of such efforts. These efforts manifested in many different ways, from redeploying healthcare staff[5] to building new temporary hospitals in a matter of days/weeks.[6] Most countries have introduced compulsory partial or total lockdown. In Ireland, the government instigated total lockdown to try to slow the spread of COVID-19 in March 2020.[7] The lockdown included: asking people to stay at home, closure of schools and universities and nonessential facilities (excluding supermarkets and health care utilities), rationing of supplies, requisition of facilities, deferral of international travel, limitation of internal journeys, and, primarily, reprioritisation of healthcare services.[8]

From this lockdown initiation date, the number of accidents related to outdoor activities were expected to decline. The Royal College of Surgeons of Ireland (RCSI) published new guidance for surgical prioritisation. It classified surgeries into four categories according to their level of urgency: priority Level 1a refers to an emergency that requires surgery within 24 hours; priority Level 1b was classified as an urgent operation needed within 72 hours; priority Level 2 was surgery that can be deferred for up to 4 weeks; priority Level 3 surgery could be delayed for up to 3 months; and priority Level 4 refers to surgery that could be delayed for >3 months.[9]

Consequently, all elective cases were postponed, limiting the demand on healthcare resources and keeping hospital beds available, if needed, for emergency admissions. Other countries such as Singapore, Australia, and New Zealand employed different approaches with the same underlying principles that were based on clinical urgency, patient and healthcare worker protection, and conservation of healthcare resources.[5,10] Australia implemented a similar but slightly less strict nationwide response on the 29th March 2020.[11] The relationship between this COVID-19 pandemic and orthopaedic surgery appears disparate when compared to other medical specialities such as infectious diseases, emergency medicine, and internal medicine. However, as part of the larger healthcare ecosystem, orthopaedic surgeons also have a role in this pandemic. This study was conducted to measure the effect this crisis was having on the trauma and orthopaedic (T&O) departments in the Republic of Ireland, to conclude whether the departments' response was effective.

PATIENTS AND METHODS

The number of patients admitted to the T&O department at Cork University Hospital (CUH), Cork, Ireland and the South Infirmary Victoria University Hospital (SIVUH), Cork, Ireland between the 1st of March 2020 and the 15th of April 2020 were recorded and this number was compared to the patient data from the same time period in 2019. All consecutive patients under consultation for T&O and requiring urgent care at CUH and the SIVUH during the lockdown period (case group) and the equivalent period in 2019 (control group) were included.

CUH is a Level 1 trauma centre and it is the only hospital in Cork that manages T&O. The city of Cork covers a total surface area of 820 km^2 in South Ireland. The population density is approximately 3,300 individuals per km^2 in the city, and this density drops to approximately 480 per km^2 when looking at the metropolitan area. The current population is 417,211, with 206,953 males and 210,258 females.[12] Typically, pre-pandemic, all trauma surgeries were performed in two operating rooms (OR) at CUH daily, except for weekends and public holidays when only one OR was available. SIVUH was used solely for elective cases. Since the advent of the crisis, CUH has only one dedicated OR for T&O surgery that is kept exclusively for elderly patients who are medically unwell, which mostly includes patients with hip fractures. The reasoning behind this approach was based on the literature that documented that older patients with medical comorbidities were more adversely affected by COVID-19 infection owing to their low functional reserves and weakened immune systems.[13-15]

The inclusion period for the case group corresponds to the confinement period in Ireland (1st March 2020 to 15th April 2020). The inclusion period for the control group corresponds to

the same time period in 2019. All patients with prosthetic joint infections which were not related to outdoors activities were excluded, as well as all spine trauma cases, as they were all either treated by the neurosurgery team or transferred to the local spinal unit in Dublin, Ireland.[16] The type of treatment (conservative or surgical) and the hospitalisation procedures (outpatient or conventional hospitalisation) were also reported.

Data Collection

All demographic data, medical history, and clinical and radiological data at presentation was collected. All trauma injuries requiring urgent care were divided into two categories: upper and lower limb. The anatomical location of the injury was also recorded so that it could be compared.

Statistical Analyses

Continuous variables are presented as mean with standard deviation; categorical variables are presented as count (%). Independent two-sample t-test and Chi-square test were used, as appropriate, to compare groups. Statistical significance was defined as $p<0.05$.

RESULTS

Lower Limb Trauma

The total number of lower limb trauma cases dropped by 20.44% from 137 (females: 87; male: 50) to 109 (females: 74; male: 35; $p=0.72$). Of note, there was a 30.00% reduction in the number of male cases (from 50 to 35; $p<0.05$), in comparison to the 14.94% reduction observed in the female population (from 87 to 74; $p<0.05$).

Upper Limb Trauma

The total number of upper limb trauma cases increased by 0.14% from 90 (female: 44; men: 46) to 94 (female: 54; male: 40; $p=0.14$). The increase reported was only applicable to the female population; the number of male cases of upper limb trauma slightly decreased from 46 to 40 ($p<0.005$).

A breakdown of operative numbers and population data can be found in Tables 1 and 2 and Figures 1 and 2.

DISCUSSION

As the current health crisis was unprecedented, no one was able to predict the consequences of such a lockdown on hand and upper extremity emergencies. Many studies have proposed guidelines to adapt the organisation of care services to cope with this outbreak, but to our knowledge, this review is the first to analyse the direct impact of this pandemic and the response to trauma emergency aetiologies and management. Some authors have highlighted a definite change in the aetiologies of injuries, which feature almost no work-related injuries and less road traffic and leisure accidents but a significant increase in domestic accidents.[17] Many patients occupied themselves with gardening, cooking, and handiwork. To some extent, it is possible to compare these results to those observed during special events such as the annual Super Bowl in the USA. People are used to staying at home, watching TV and cooking, temporarily living a very short experimental lockdown.[18]

Additionally, the lockdown has had many psychological impacts on the population, with an increase in domestic violence and anger accidents. This extraordinary social situation has led to an increase in the level of stress and anxiety of entire populations, starting with the patients who had to consult with the hospital. The mental health effects of the COVID-19 pandemic might be profound.[19] Interventions to address the psychological and social long-term repercussions of the pandemic are required.[20] Moreover, these results confirm a considerable decrease in the rate of lower limb trauma compared to the reference period in 2019, as well as many other emergencies. For example, in some countries, the number of acute stroke admissions dropped by 50%.[21] This reduction in activity has enabled the relocation of surgeon teams to other sectors. All over the world, different actions were put in place in response to the COVID-19 pandemic by orthopaedic surgeons.[22-24]

In this study, formalised patient care was created at the hospital level. Upon arrival in the emergency room, suspected or confirmed COVID-19 patients followed a circuit sealed from COVID-19 negative patients.[25]

Table 1: Comparison between the numbers of patients undergoing surgery at Cork University Hospital, Cork, Ireland and South Infirmary Victoria University Hospital, Cork, Ireland in March–April 2019 and 2020.

Number of surgeries	Control group	Case group	Percentage change	p value
Lower limb	137	109	-20.44%	0.72
Upper limb	90	94	+4.44%	0.14

Table 2: Patient sex.

	Sex (female/male)		p value
	Control group	Case group	
Lower limb	87/50	74/35	0.001
Upper limb	44/46	54/40	0.007

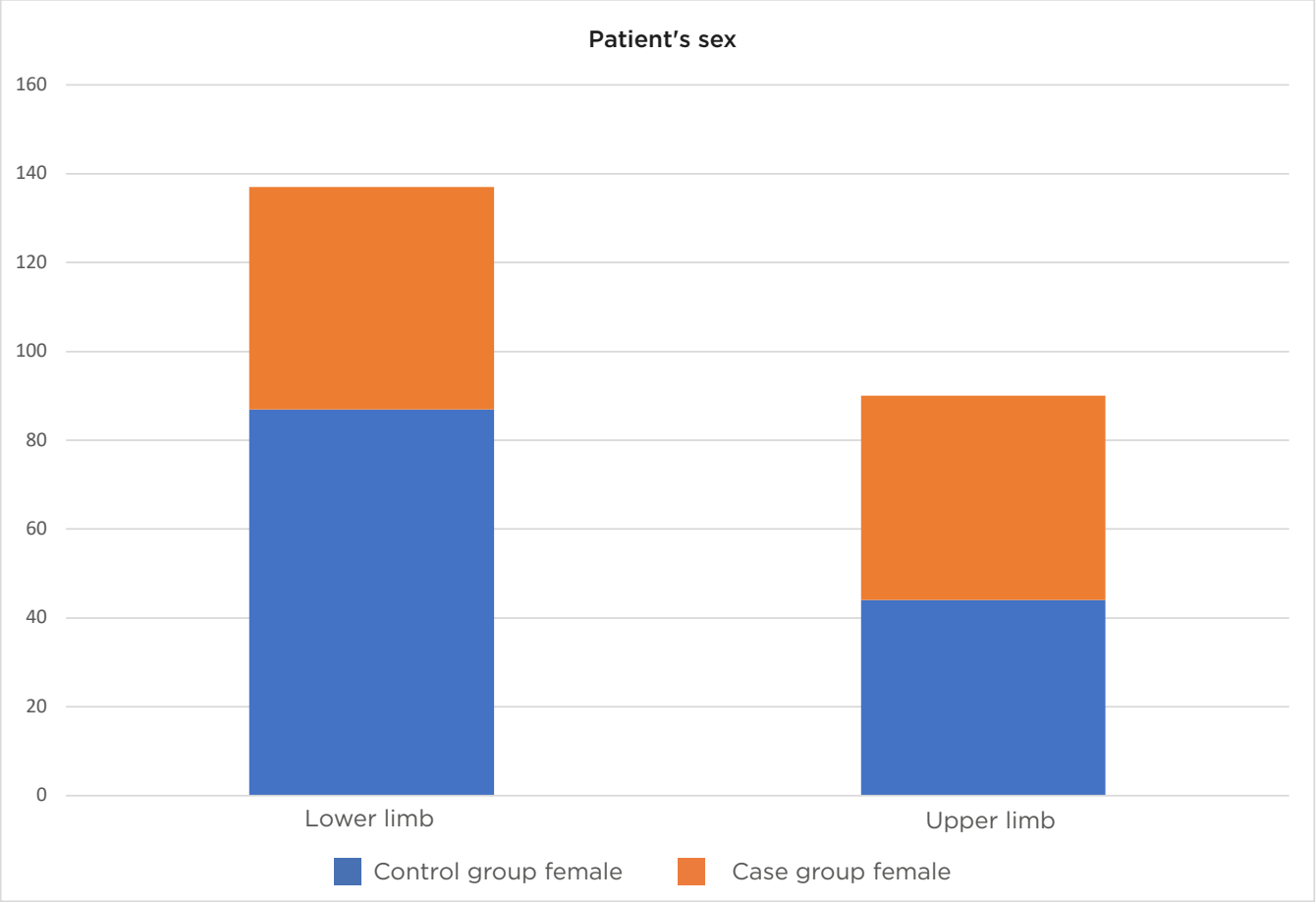

Figure 1: The incidence of trauma according to the patient's sex.

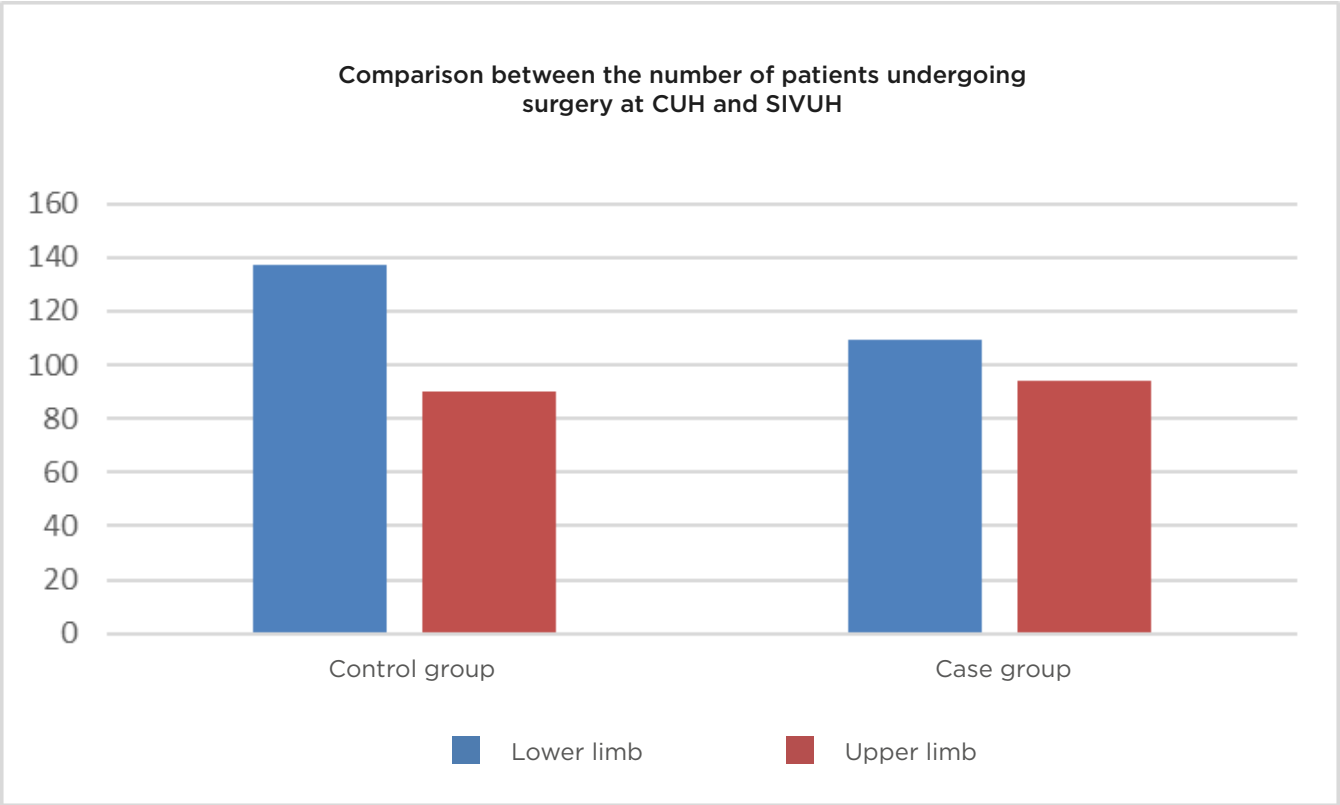

Figure 2: Comparison between the case group and a control group.

CUH: Cork University Hospital; SIVUH: South Infirmary Victoria University Hospital.

With the limitation of log-on to operating rooms and anaesthetic backing, indications for conservative treatment were driven to the maximum. Anaesthetic procedures like wide-awake local anaesthesia no tourniquet (WALANT) have been proposed to continue the care of patients with trauma injuries without an anaesthetic team and crucially without generating aerosolised particles which could put healthcare workers lives' at higher risk.[17] Indeed, nursing staff and orthopaedic and anaesthetic teams are known to have been infected with COVID 19.[26] In this study, protection rules to adequately protect health surgeons were applied.[27,28] Unfortunately, three patients with confirmed COVID-19 operated in this study cohort were unable to benefit from this technique. Despite the risks involved and the organisational constraints, staff treated all patients optimally without reducing the level of quality care.

This pandemic happened in the digital age and has hugely changed working habits. In particular, new services such as telehealth, virtual visits, and online tools for postoperative rehabilitation have been developed.[29,30] These technologies will accompany virtual and enhanced reality training and digitalisation of meetings in the healthcare system in the future.

In the author's department, the hospital authorities increased the number of inpatient beds from 66 to 90 and the number of operating rooms from two to three. Additionally, all elective surgeries were cancelled to preserve valuable equipment, nurses, and essential staff resources to treat the virus.[31] The COVID-19 crisis impacted the numbers of T&O operations performed in a Level 1 major trauma centre in the South of Ireland, but not to a great extent; only a 10.15% decline was observed.

Because individual sporting activities like running and cycling were not prohibited, the numbers of lower limb trauma actually decreased by 20.44%, possibly because these fracture types require high-impact trauma,[32] accounted for mostly by road traffic accidents.[8]

The number of females requiring T&O Surgery

between the two time periods observed was increased, and any reduction in overall numbers was accounted for by the fall in the number of male patients only; down from 96 to 75, illustrating a 21.87% decrease.

CONCLUSION

The COVID-19 crisis has led to a decrease in the total numbers of lower limb trauma surgeries. However, the upper limb fractures all increased. The main reduction was among the male patients, whilst the number of females presenting with upper limb fractures increased.

STUDY LIMITATION

The author acknowledges that a limitation of this study is the collection of data from one centre only. Multicentre studies with larger cohorts are warranted to add to the growing body of literature documenting the impact of COVID-19 on T&O surgery not only locally, but internationally.

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Implications and Aspects of Lyme Neuroborreliosis

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Abstract

Lyme borreliosis or Lyme disease affects thousands of people globally each year, and, with nervous system involvement, this disease can lead to the development of Lyme neuroborreliosis (LNB). If not diagnosed and treated properly, LNB can lead to serious life-long health implications for affected patients. The clinical manifestations and treatment regimens are relatively well-studied, but much remains unknown about the disease's pathogenesis and epidemiology. In this review, the authors elucidate the knowns and unknowns of LNB.

INTRODUCTION

Lyme borreliosis, also known as Lyme disease, is an emerging infectious disease that has gained global attention.[1] The name Lyme disease stems from Old Lyme, Connecticut, USA, where in the 1970s a cluster of oligoarthritis was observed in children who lived in rural environments.[2,3] Subsequent investigations led to the true cause: spirochaetes of the genus *Borrelia*, which are transmitted via ticks of the genus *Ixodes*:[2] *Ixodes scapularis* and *Ixodes pacificus*, found in the eastern and western parts of North America, respectively; and *Ixodes ricinus* and *Ixodes persulcatus* in Europe and Asia. The tick has a three-stage life cycle, which includes larval, nymph, and adult stages.[2,4] Ticks in the larval stage will initially feed on a variety of animals that are reservoirs for spirochaetes, which include rodents, birds, and other small vertebrates. The larvae will acquire the spirochaete from the infected host.[2,4] After developing into the nymphal life-stage, the tick will often feed on similar species as the larval stage, thus transmitting the spirochaete to a new host, resulting in a cycle of infection (Figure 1).[5] The nymphs may also feed on larger animals, including dead-end hosts, such as humans; nymphs are responsible for the majority of cases of Lyme disease in humans.[1,2] Adult ticks primarily feed on larger animals such as deer, which are not considered competent hosts of *Borrelia* species but serve as a primary location for ticks to mate.[6]

Lyme borreliosis is the most common vector-borne disease in North America.[1] Cases in the USA have increased from 10,000 annual cases in 1991 to >25,000 in 2014;[1] however, the true number of infected individuals is thought to be under-reported by as much as 10-fold.[7,8]

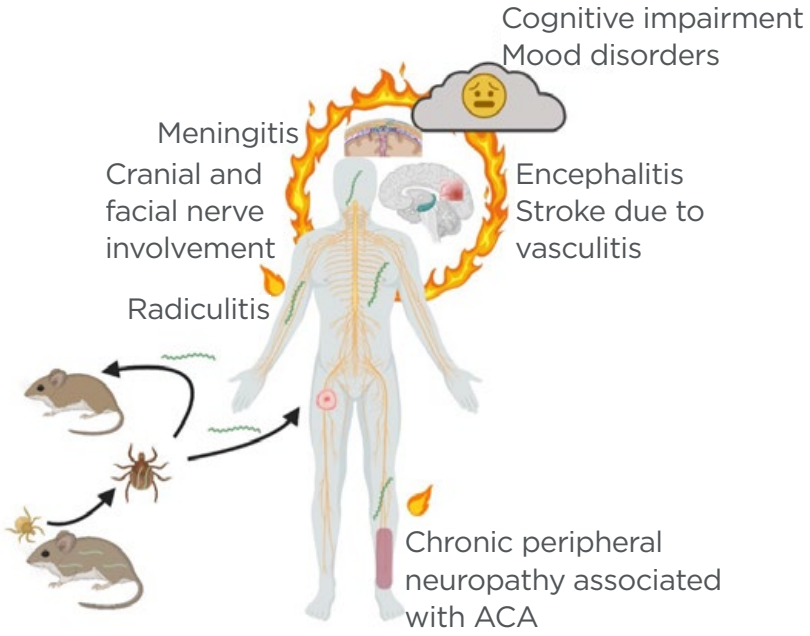

Figure 1: *Borrelia* life cycle and development of Lyme neuroborreliosis.

Larval ticks are uninfected as there is no trans-ovarial transmission of Lyme borreliae. Larval ticks feed on small vertebrates such as mice; if the tick feeds on an infected animal, it will acquire the spirochaete. Upon molting to the next life stage, the nymphal tick will again seek a blood meal, again from small animals or birds. If the tick feeds on an uninfected host, it will transmit the spirochaete to the reservoir host, thus maintaining the Lyme borreliae in nature. Nymphs will also feed on larger, dead-end hosts such as humans; indeed, the greatest risk of human infection with Lyme borreliae is through the bite of a nymphal tick. The spirochaetes disseminate from the initial bite site, where the characteristic erythema migrans rash occurs. Spirochaetes disseminate widely throughout the body and affect many tissues and organ systems including the joints, heart, and central and peripheral nervous systems. Nervous system involvement may include Lyme meningitis, radicular pain, and involvement of the cranial nerves, resulting in a temporary and partial paralysis of one side of the face (Bell's palsy). Less common but more serious manifestations include encephalitis or cerebral vasculitis. Long-term sequelae may occur, and many patients experience fatigue, mood changes, difficulty sleeping, and deficits in short-term memory or 'brain fog'. European patients with the late manifestation of acrodermatitis chronica atrophicans may experience chronic polyneuritis or neuropathy.

ACA: acrodermatitis chronica atrophicans.

Created with BioRender.[5]

In the USA, cases are most prevalent in the Midwest and Northeast.[9] In Europe, Lyme borreliosis is most prevalent in Scandinavian countries and central Europe, with total estimates ranging from 65,000 to >160,000 per year.[10,11] For example, in Germany there are approximately 30,000 cases annually, with some sources estimating the true number of annual cases to be 200,000.[12] Lyme borreliosis in North America and Europe will likely continue to increase due to population growth, humans living in infected tick habitats, and climate change. Some studies predict that climate change will result in a 21% increase of Lyme borreliosis cases in the USA, as recorded temperature trends in the USA point to a probable increase of 2 °C by mid-century, resulting in a more stable and suitable environment for infected ticks.[13]

When the tick bites a human, it transmits spirochaetes with its saliva, resulting in a focal infection in the skin and distal systemic infections after 1–2 weeks.[1,14,15] The spirochaetes responsible for Lyme borreliosis are collectively known as *Borrelia burgdorferi sensu lato* (*Bbsl*). Amongst 20 different species, there are three responsible for most disease seen in humans:[2] in North America, *Borrelia burgdorferi sensu stricto*; in Europe, primarily *Borrelia afzelii* and *Borrelia garinii*.[2,7,14,16] The focal infection results in a visible, expanding skin rash known as erythema migrans in 60–80% of Lyme borreliosis cases in

the USA;[14,15] erythema migrans is also the most common manifestation of Lyme borreliosis in Europe.[2] Different genospecies of *Bbsl* cause differing signs and symptoms in their infected hosts.[2,12,17,18] For example, European patients may develop a late-stage skin disorder called acrodermatitis chronica atrophicans (ACA), which is not typically seen in North American patients.[17,18] Other clinical manifestations of Lyme borreliosis arise from the effects of the disease on the heart, joints, and nervous system (Figure 1).[5,16,19]

CLINICAL MANIFESTATIONS OF LYME NEUROBORRELIOSIS

The course of Lyme borreliosis involves three stages: early localised, early disseminated, and late disseminated stages.[2,14] The early localised stage is characterised by erythema migrans. The rash typically presents at the site of inoculation, 1–2 weeks after infection.[2,14] The early disseminated stage usually starts 3–5 weeks after the initial tick bite and is characterised by the development of multiple secondary erythema migrans and cardiac and neurologic manifestations, typically facial nerve palsy, polyradiculitis, and meningitis.[14,16,17] Lyme arthritis, ACA, and neurologic manifestations may occur during the late disseminated stage.[14] Neurologic manifestations, both early and late, can involve the central and peripheral nervous system[19,20] and are collectively known as Lyme neuroborreliosis (LNB).[12] Approximately 3–15% of patients develop LNB.[12,16,21] LNB may develop weeks to months after the host has been infected, and typical manifestations are peripheral, often bilateral, facial nerve palsy, polyradiculitis, and meningitis. Atypical presentations include myelitis or central nervous system (CNS) vasculitis.[22]

The first case of neuroborreliosis described in the literature involved a 58-year-old French male in 1922 who developed a large erythroderma after a tick bite.[22,23] The man experienced bilateral radicular pain that eventually led to muscle weakness and atrophy. Analysis of his cerebrospinal fluid (CSF) revealed pleocytosis and elevated protein levels. A triad of meningitis, radiculoneuritis, and cranial neuritis encompasses the more common clinical manifestations of LNB.[22] Also, over half of the patients that experience radiculitis have symptoms that involve cranial nerves, more commonly the facial nerve, which can be unilaterally or bilaterally affected.[12] Peripheral nervous system (PNS) involvement can also occur; this is particularly problematic as PNS manifestations can mimic other disease states.[24] A recent study characterised a small cohort of patients with PNS involvement, documenting meningoradiculoneuritis with distal weakness and loss of sensation, axonal neuropathy with parasthesia and pain, and acute demyelinating neuropathy.[24] As symptoms can be reversed with timely antibiotic therapy, it is crucial to be alert for PNS involvement.

Notably, there are regional differences for clinical manifestations between European and USA patients because of the different genospecies of *B. burgdorferi* causing infection. In Europe, the predominant species causing LNB include *B. garinii* and *B. afzelii*, while in the USA infection is predominately caused by *B. burgdorferi sensu stricto*.[12] Adults in European countries with LNB caused by *B. garinii* typically experience a painful radiculitis with an asymmetric onset and lymphocytic meningoradiculitis (also known as Bannwarth syndrome), which lasts for weeks to months and is exacerbated at night-time.[16,20] Segmental pain, often on the extremity where the tick bite occurred, appears first; this pain is intense and fails to respond to typical analgesics.[25] Cranial nerve deficits often follow.[25] Those infected with *B. afzelii* experience a broader range of symptoms that may include cognitive dysfunction and dizziness.[12,20] Children and adolescents in Europe affected with LNB commonly present with lymphocytic meningitis.[12] The most common clinical manifestation of LNB in the USA is subacute aseptic meningitis with an onset within weeks to months after tick bite.[16,19] Facial palsy is also common.[12] Children with LNB in the USA differ from their adult counterparts as the most common clinical manifestation is peripheral facial nerve palsy, rather than subacute aseptic meningitis.[19,20] If a patient still has neurological manifestations for 6 months or longer, this is considered 'late Lyme neuroborreliosis'. [12] Late LNB can occur

months to years after being infected. Only a small proportion of patients with LNB go on to develop late LNB, characterised by chronic meningitis, progressive encephalitis, myelitis, or cerebral vasculitis.[12] Polyneuropathy or polyneuritis as a late consequence of LNB can occur in association with ACA in European patients.[25]

PATHOGENESIS OF LYME NEUROBORRELIOSIS

Spirochaetes elicit an inflammatory response within the CNS including pro-inflammatory cytokines and chemokines and recruitment of immune cells.[26-28] Unlike acute bacterial meningitis, patients with LNB present with a lymphocytic, rather than neutrophilic, pleocytosis in the CSF.[29] B lymphocytes are recruited, become activated, and mature into plasma cells.[30] One diagnostic criterion of Lyme neuroborreliosis is the presence of *Borrelia*-specific antibodies in the CSF.[31] The key role for inflammation in the pathogenesis of LNB is exemplified by studies involving dexamethasone treatment of rhesus macaques infected with *B. burgdorferi*. Animals treated with dexamethasone, a corticosteroid with anti-inflammatory properties, had significantly reduced levels of lymphocytic pleocytosis and immune mediators in the CSF.[32] The dexamethasone-treated macaques also had no inflammatory lesions in the brain and spinal cord, no signs of focal neurodegeneration and demyelination in the spinal cord and peripheral nervous system, and a significant reduction in neuronal and glial cell apoptosis in the dorsal root ganglia.[32] Markers of inflammation remained elevated in the infected macaques that were left untreated. There are several potential mechanisms for the neurological manifestations induced by *Bbsl* infection, including direct cytotoxicity, neurotoxic mediators, and production of autoantibodies.[30,32,33] In addition to inducing an inflammatory response, spirochaetes bind to proteoglycans and/or galactocerebrosides on neuronal and glial cells in the CNS as well as Schwann cells of the PNS[32] and can induce apoptosis. *Bbsl* do not produce or secrete toxins, but rather can induce the host's cells to secrete neurotoxic agents such as nitric oxide.[30,32,33] Nitric oxide can then cause neuronal damage either directly, by inducing the production of pro-inflammatory cytokines, or by promoting vascular permeability.[34] Another example is *Bbsl*-induced secretion of quinolinic acid; this neurotoxin is secreted by macrophages and causes neuronal death at high concentrations.[30,33] *Bbsl* infection can also induce the formation of auto-antibodies that attack components of the host's nervous system.[30,33] While inflammation and a deleterious host immune response are clearly implicated in the pathogenesis of LNB, much is unknown, including bacterial factors contributing to neurotropism or neurotoxicity.

DIAGNOSIS OF LYME NEUROBORRELIOSIS

Indicators of LNB vary depending on the age and geographic location of the patient. Hence, the differential diagnosis for a patient with LNB can be quite broad. A presentation of cranial neuritis or subacute aseptic meningitis with a preceding erythema migrans rash makes a diagnosis of LNB more likely.[35] Laboratory studies to confirm the possible diagnosis of LNB include methods that detect the presence of *Bbsl* and the immune response initiated against the bacteria.[19] The direct detection of *Bbsl*, either in the CSF or other accessible fluids via culture or nucleic acid detection tests, currently holds low diagnostic value.[19,35,36] Investigation of the CSF is the only way to make a definite diagnosis of LNB. CSF studies focus on three main parameters: lymphocytic pleocytosis, intrathecal antibody production, and protein content.[19,35,36] Lymphocytic pleocytosis with plasma cells, with an average cell count of 200 cells/mL, is an important cellular marker for the diagnosis of LNB as the presence of lymphocytes can help rule-out other neurological disorders.[25,35] The CSF also usually exhibits an increased total protein content.[25] Intrathecal IgM synthesis occurs in almost all patents with early manifestations, and is an important parameter but must be measured with caution because antibodies specific for *Bbsl* produced in the periphery can enter the CSF.

Therefore, it is paramount that CSF antibody titres are measured in relation to the antibody titres from the serum from samples collected at the same time to determine the *Borrelia*-specific antibody index.[19,36] Blood–CSF barrier function must be taken into account; a recommended calculation[25] is:

$$\text{antibody index} = \frac{\text{specific IgG Ab CSF (units):spec.IgG Ab serum (units)}}{\text{IgG concentration CSF (mg/mL):IgG concentration serum (mg/mL)}}$$

Serum studies are used to detect antibody production specific for *Bbsl*. In the USA, the Centers for Disease Control and Prevention (CDC) currently recommends a two-tier approach when testing serum from patients with suspected LNB.[19] First, serum samples are screened by ELISA and positive or borderline-positive results are followed with separate immunoblots for *Bbsl*-specific IgM and IgG.[19] The two-tier approach has significant limitations, particularly in diagnosis of early disease, as there is a significant time lag between tick bite and specific antibody production.[11] A cellular marker that shows promise for the detection of LNB is chemokine ligand 13 (CXCL13).[37] CXCL13 is a chemoattractant for B lymphocytes, and CXCL13 concentrations are elevated in the CSF during *Bbsl* infections.[19,36,38] Studies have also indicated that CXCL13 can be used as a cellular marker for active spirochaetal infection, as when patients with LNB are treated their CXCL13 concentrations drop significantly. However, CXCL13 is not specific for LNB and can be elevated in the CSF during other disease processes. The utility of CXCL13 during the diagnosis and management of LNB is still being determined but may be useful in monitoring treatment response.[19,37,39] Imaging studies such as MRI may be useful to rule out other neurological conditions.[40,41] Electrophysiology studies, to measure compound muscle action potentials as well as abnormalities of F-waves or of conduction, can help differentiate between axonal and demyelinating neuropathies.[24]

TREATMENT OF LYME NEUROBORRELIOSIS

Treatment for LNB, with an oral or parenteral regimen, is dependent on the region where the patient was infected or lives and their disease pathology. If the patient is to be treated orally, doxycycline has the appropriate pharmacokinetics and pharmacodynamics. Doxycycline, when given daily in 200 mg doses, can reach concentrations in the CSF that are able to eliminate many *Bbsl* strains,[42] because of doxycycline's high lipid solubility, which allows the drug to easily pass through the blood–brain barrier.[43] It is recommended to treat the patient for 21 days with doxycycline in order to reach a steady-state equilibrium. Some studies have shown that giving patients a daily 400 mg dose of doxycycline is more effective for treating LNB compared to the daily 200 mg dose, as effective CSF concentrations can be reached faster and the treatment duration can be shortened.[44] The oral regimen with doxycycline for treating LNB is commonly used in European countries.[31,42] Patients in the USA with LNB are typically treated with ceftriaxone parenterally for 10–14 days.[44] European researchers have demonstrated that *B. burgdorferi sensu stricto*, the spirochaete that causes the majority of LNB cases in the USA, is susceptible to oral doxycycline.[43] In addition, doxycycline is found in similar concentrations in the blood of treated patients, whether it is given orally or intravenously.[45] Despite the support for an oral treatment of LNB with doxycycline, the parenteral regimen may be preferred in cases, such as where there is evidence of parenchymal involvement.[45] Lastly, while it is imperative to eradicate the spirochaetes with antibiotics, decreasing inflammation with non-steroidal anti-inflammatory drugs[46] may also be efficacious. Treatment recommendations for adult and paediatric patients at distinct stages of LNB are summarised in Table 1.[47]

CONTROVERSIES RELATED TO LYME NEUROBORRELIOSIS

The term 'chronic' can refer to an illness persisting for a long time.[48] In terms of Lyme borreliosis, the term has been applied both to late-stage manifestations of the disease (e.g., ACA),[18] as well as a catch-all term for patients suffering from a multi-system dysfunction with prolonged fatigue, pain, and neurological issues.[49] Chronic Lyme disease (CLD) is a controversial diagnosis that can encompass a variety of patient populations.

Table 1: Treatment doses for Lyme disease and Lyme neuroborreliosis.

	LD	Early LNB	Facial palsy*	Meningitis/ radiculoneuritis*	Late LNB
Adult doses					
Doxycycline	200 mg daily for 10–14 days	200 mg daily for 14–28 days	100 mg twice daily for 14–21 days	200 mg daily divided into 1–2 doses for 14–21 days	200 mg daily for 28 days
Ceftriaxone	2 g daily for 14 days	2 g daily for 14–28 days	NR	2 g intravenously once daily for 14–21 days	2 g daily for 14–28 days
Paediatric doses					
Doxycycline	Two doses of 4.4 mg/kg for 10–14 days (max. of 100 mg/dose)	Two doses of 4.4 mg/kg for 14–28 days (max. of 100 mg/dose)	4.4 mg/kg per day orally, divided into 1–2 doses for 14–21 days (max. 100 mg/dose)	4.4 mg/kg per day orally, divided into 1–2 doses for 14–21 days (max. 100 mg/dose)	Two doses of 4.4 mg/kg for 28 days (max. 100 mg/ dose)
Ceftriaxone	50–100 mg/kg for 14 days (max. 2 g/ dose)	50-100 mg/kg for 14-28 days (max. 2 g/dose)	NR	50–75 mg/kg intravenously once daily for 14–21 days (max. 2 g/dose)	50–100 mg/kg for 14–28 days (max. 2 g/dose)

*Centers for Disease Control and Prevention (CDC) Neurologic Lyme recommendations.[47]
LD: Lyme disease; LNB: Lyme neuroborreliosis; max.: maximum; NR: not recommended.
Adapted from Stanek and Strle.[17]

These patient populations include patients diagnosed with post-treatment Lyme disease syndrome (PTLDS), patients experiencing prolonged objective symptoms of Lyme disease, and patients with unexplained clinical manifestations that have not previously been diagnosed with Lyme borreliosis.[50-53] The Infectious Diseases Society of America (IDSA) provides a working definition for PTLDS as symptoms in patients who have been previously appropriately treated for Lyme borreliosis and remain symptomatic for at least 6 months, with subjective symptoms including fatigue, pain, and cognitive issues that are severe enough to interfere with work, school, and life.[51,54,55] The International Lyme and Associated Diseases Society (ILADS) has recently provided a definition for CLD, but the definition is broad and still creates challenges for excluding CLD from other disorders or syndromes.[52] ILADS defines CLD as a multi-system illness with a wide range of symptoms present for a minimum of 6 months, either intermittent or continuous, and includes subcategories: untreated CLD and previously treated CLD. Many of the patients diagnosed with CLD experience the following symptoms: fatigue, myalgias, arthralgias, headaches, and subjective cognitive dysfunction.[50,51] Notably, many of those symptoms overlap with the experiences of patients with PTLDS. Some patients referred for CLD are found to be experiencing an unrelated neurologic, psychiatric, or rheumatologic condition.[50,51] The picture is complicated by patients with a previous Lyme borreliosis diagnosis that experience residual symptoms for months after treatment. As with other infections, some patients will commonly experience prolonged fatigue after their infection resolves. Prolonged

fatigue, along with symptoms such as chronic pain, are relatively frequent findings, even in the general population.[50] Differentiating between these symptoms is a dilemma for clinicians, especially when deciding whether to treat with long-term antibiotic therapy. Several studies have concluded that further antibiotic use for patients experiencing prolonged Lyme borreliosis symptoms is not beneficial and may be harmful to the patient.[50,51,53] Adverse events that have occurred include catheter-associated venous thromboembolism, catheter-associated bacteraemia, and allergic reactions.[53,56] A clinician will ideally examine the patient with a holistic assessment, being alert to other diagnoses while remaining sensitive to the patient's symptoms and concerns. This is particularly important as many of these patients feel ignored or abandoned by the medical establishment and may turn to alternative sources of information. Unfortunately, self-diagnosis of CLD has contributed to the death of a patient.[57] It is imperative that clinicians work to build a sense of trust with the patient to combat the all-too-common dissatisfaction with the medical community from patients with CLD symptoms.[49,51-53]

CONCLUSION

Lyme borreliosis is caused by a spirochaetal infection, which can involve the nervous system. With cases increasing significantly on a global scale, the importance of continued research into LNB is evident. Future research into LNB should focus on aspects of borrelial pathogenesis and the epidemiology of the disease, as well as on the development of more effective diagnostic tools for this serious disease.

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Disseminated Phaeohyphomycotic Lymphadenitis with *Cladophialophora* Species

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Abstract

Objective: This study describes a case of disseminated phaeohyphomycotic lymphadenitis in a young female with delayed diagnosis and good clinical response after appropriate treatment.

Methods: A 32-year-old female presented with erythematous to violaceous papules with oozing discharge bilaterally in her inguinal region for a few months. History revealed tuberculous meningitis 4 years earlier treated with first-line anti-tuberculous therapy for 18 months, and 2 years previously she developed pigmented discharging lymph nodes bilaterally in her axillae. The histopathology of the biopsy of the axillary nodes showed chronic granulomatous inflammation with multiple branching septate fungal hyphae. She received amphotericin B for 21 days but without improvement. Biopsy from the inguinal lesions was sent for histopathology and culture.

Results: Histopathology of the biopsy material showed chronic granulomatous inflammatory process with multinucleate giant cells, epithelioid cells, histiocytes, and lymphocytes with multiple branching septate fungal hyphae. Gram stain revealed moderate septate hyphae with numerous pus cells. Culture on Sabouraud dextrose agar yielded velvety olive–black colonies in the fourth week. Microscopic slide examination of culture material was suggestive of Cladophialophora species. The patient was started on voriconazole, which was continued for 6 months, and showed clinical improvement.

Conclusion: Incomplete investigation of infectious lesions may delay diagnosis. Furthermore, clinical presentations are greatly influenced by the immune status of the host. Both histopathological and microbiological assessments are equally important for making a conclusive diagnosis. Anti-fungal therapy may delay the growth of fungi that normally grow within a week; thus, a longer incubation time may be warranted for fungal smear-positive samples.

INTRODUCTION

Phaeohyphomycosis is an infection of the skin, subcutaneous tissues, and internal organs caused by dematiaceous (melanised) fungi found in soil, organic material, plants, and air. Histopathology of infected tissues usually reveals dematiaceous septate, regular or distorted, toruloid hyphae beside yeast-like cells. The presence of melanin in their cell walls is considered a virulence factor for these fungi. During the past several decades, phaeohyphomycosis has been attributed to over 100 species and 60 genera of fungi. Most clinically relevant species are within the genera of *Scedosporium, Exophiala, Cladophialophora, Exserohilum, Bipolaris*, *Curvularia*, *Wangiella,* and *Lecythophora.*[1] Although cerebral infection is the most serious form of systemic phaeohyphomycosis, other localised deep forms of the disease have been reported.

An infection is likely introduced by a traumatic inoculation, which may even be unrecognised by the patient, or inhalation of the aetiologic agent.[2] Skin and subcutaneous tissue involvement are termed subcutaneous phaeohyphomycosis, which is characterised by papulonodules, verrucous, hyperkeratotic or ulcerated plaques, cysts, abscesses, pyogranuloma, and non-healing ulcers, or sinuses. Clinical presentations are also influenced by the immune status of the host. The primary risk factor is decreased host immunity, although cases in apparently immunocompetent patients have been reported.[2,3] Phaeohyphomycoses are frequently associated with debilitating chronic diseases like diabetes, rheumatoid arthritis with prolonged corticosteroid therapy, and solid organ, especially kidney, transplant.[4] Moreover, long-term immunosuppression from corticosteroids may lead to delayed clinical expression.[5] While immunocompromised patients are at risk of locally invasive phaeohyphomycosis, rarely fatal complications such as pneumonia or disseminated disease may occur. Disseminated phaeohyphomycosis is an uncommon infection, although its incidence may be increasing in the population.[1] Early diagnosis and careful interpretation of culture results are therefore important for the treatment of these patients. This communication reports a case of subcutaneous phaeohyphomycosis with disseminated lymphadenitis caused by *Cladophialophora* species.

CASE

A 32-year-old female, with a history of treated tuberculous meningitis in 2014, presented with erythematous to violaceous papules, with oozing discharge bilaterally in her inguinal region for a few months. She reported that in March 2016 she had developed similar pigmented discharging lymph nodes bilaterally in her axillae and slight enlargement of her left inguinal lymph node with minimal discharge. The axillary lymph nodes biopsy at the time showed ulcerated skin with underlying adipose tissue exhibiting dense acute and chronic inflammatory infiltrate. Focal abscess formation and necrosis with multiple branching septate fungal hyphae were also highlighted by periodic acid–Schiff and diastase special stain. The patient did not recall any fever or systemic symptoms and her erythrocyte sedimentation rate was 5 mm/hour at that time. She was non-reactive for HIV. She received amphotericin B for 21 days for the axillary lesions, with no response. However, in March 2018 she developed new lymph nodes. Biopsy showed non-specific inflammation. Another trial of amphotericin for 21 days was given, which failed again. Subsequently, another biopsy was taken in October 2018, this time from inguinal lesions (Figure 1A), and it was sent for histopathology and fungal culture. Histopathology revealed chronic granulomatous inflammation with multinucleated giant cells, epithelioid cells, histiocytes, and lymphocytes with multiple branching septate fungal hyphae, suggesting a deep-seated fungal infection (Figure 1B). Special stain periodic acid–Schiff with or without diastase also revealed fungal spores and hyphae. Acid-fast bacilli and Wade-Fite stain (for *Mycobacterium leprae*) were both negative.

Gram stain showed moderate pigmented septate hyphae with numerous pus cells. In the fourth week of incubation at 37 °C, culture on Sabouraud dextrose agar yielded velvety olive–black colonies with a black reverse (Figure 1C). Microscopic slide examination of the culture on Sabouraud dextrose agar revealed dark, septate, freely branching hyphae giving rise to medium-length chains of dark staining conidia. The conidia were elliptical in shape, smooth with a prominent hilar scar, and 10–15 conidia-long chains arose from 'shield cells' where branching occurred (Figure 1D).

The isolate failed to grow at 42 °C while it grew well at 37 °C and 25 °C and was thus identified phenotypically as *Cladophialophora* species.[6]

The patient was initiated on voriconazole the same month, to which she gradually responded, with resolution of the axillary lesion and significant reduction in the size of the inguinal lymph node, as monitored by ultrasound. Her HIV status remained negative. Unfortunately, the patient was lost to follow-up until April 2019. During the intervening months, she had stopped the voriconazole in January 2019, leading to another relapse, and had subsequently restarted this for a month only to stop again. As she was asymptomatic, she was observed off therapy.

She developed discharge from the lesions and therapy was reinitiated in April 2019 with adherence counselling. She responded well to therapy, with a gradual reduction in the size of the nodes (as documented on ultrasound; Table 1). Voriconazole was continued up to June 2020 until stability in the lymph node size was achieved. A follow-up ultrasound is awaited. Given the severe financial constraints of the patient, a work-up for immunodeficiency could not be performed.

DISCUSSION

The authors describe a case of *Cladophialophora* species lymph node and cutaneous infection in an apparently immunocompetent host. Most clinically relevant species are within the genera of *Exophiala, Cladophialophora, Coniosporum, Cyphellophora, Fonsecaea, Phialophora,* and *Rhinocladiella.* Several cases of phaeohyphomycosis and chromoblastomycosis have been reported from India and Brazil.[7-9]

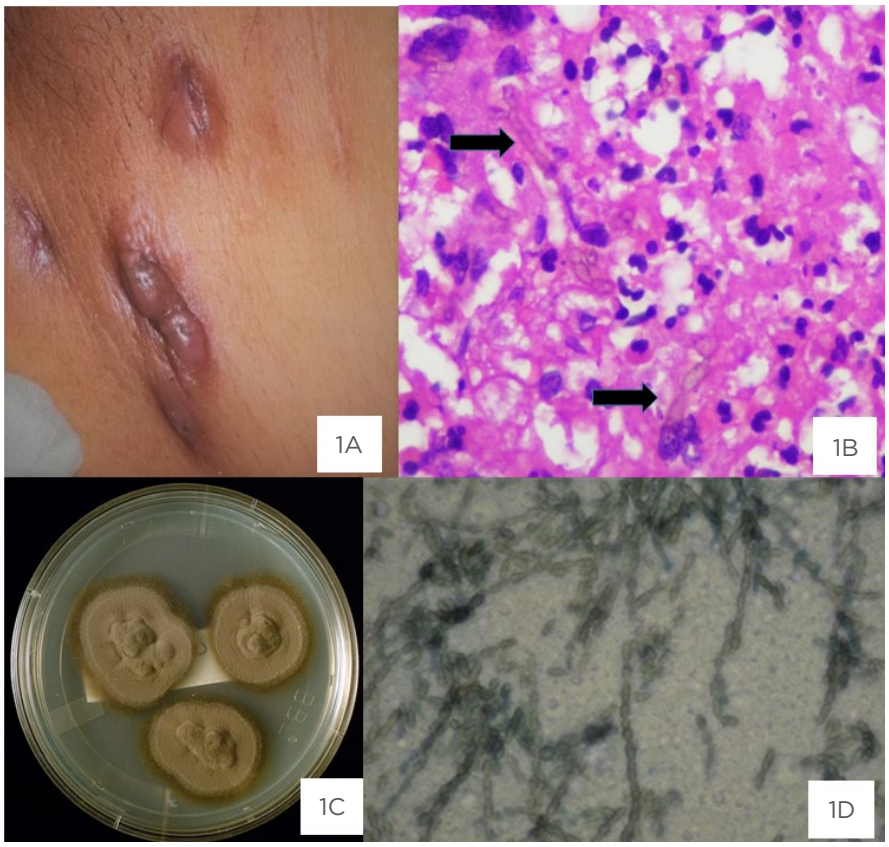

Figure 1: Image plate of clinical lesion, histopathology, macroscopic colony morphology, and microscopic appearance of the organism, Cladophialophora species. Draining sinuses in the groin and lower abdominal wall.

1A: Draining sinuses in the groin and lower abdominal wall with dark pigmented discolouration of skin. 1B: Histopathology of inguinal lymph node showing chronic granulomatous inflammation and golden brown pigmented septate hyphae with some dilated forms (arrows). 1C: Culture on Sabouraud's dextrose agar: velvety olive-black colonies at 4 weeks of incubation at 37 oC. 1D: Chains of 10–15 elliptical dark conidia with branching.

Table 1: Progression of the lymph node size on therapy with voriconazole (initiated April 2019).

Year	Left inguinal (cm)	Right inguinal (cm)
June 2019	2.7 x 1.1	2.6 x 1.0
August 2019	2.4 x 1.1	2.1 x 0.8
November 2019	2.0 x 0.8	2.1 x 0.7
February 2020	1.8 x 0.8	1.9 x 0.7
June 2020	1.4 x 0.7	1.9 x 0.7

Limited data are available from Pakistan; however, laboratory-based surveillance from the authors' institute reported cerebral phaeohyphomycosis to be as common in brain abscesses as that due to *Aspergillus* species.[10] Moreover, melanised fungi resulted in around 9% of all invasive skin and soft tissue infections in the data.[10] There is a scarcity of reported data from Pakistan; however, few studies report these infections mainly in immunocompetent patients.[11,12] One reason for the relatively higher frequency of these moulds in low-to-middle-income agricultural countries may be the humid, tropical, and subtropical climate, which potentially facilitates the growth of these organisms in the environment.[13]

The organisms are likely introduced by a minor traumatic inoculation, which is often not recognised by the patient.[3] Clinical presentations are greatly influenced by the immune status of the host. Regarding the patient in this current study, a history of tuberculosis may also suggest a poor cell-mediated immune response; therefore, a trivial, unnoticed trauma may have been responsible for initiation of the infection. Additionally, the authors did not evaluate for genetic mutations such as *CARD9* deficiency, which has been reported to predispose to serious fungal infections in otherwise healthy patients from the region.[14]

Several similar cases have been reported from Brazil. In a series of 18 cases of phaeohyphomycosis, 61% presented with subcutaneous lesions. In all of the cases, the presence of melanin in the fungal cells was determined by Fontana-Masson staining of tissue sections and documented. The causative organisms included *Exophiala jeanselmei, Alternaria, Curvularia, Cladophialophora,* and *Colletotrichum gloeosporioides.*[8]

Treatment of *Cladophialophora* species is often difficult, prolonged, and not standardised. Itraconazole and voriconazole have a broad range of activity against phylogenetically similar agents of hyalohyphomycosis, phaeohyphomycosis, chromoblastomycosis, and mycetoma. *In vitro* comparison showed that the minimum inhibitory concentration (MIC) ranges (geometric mean) of amphotericin B, itraconazole, and voriconazole against *Cladophialophora carrionii* were 0.06–4.00 µg/mL (1.07 µg/mL), 0.03–0.06 µg/mL (0.03 µg/mL), and 0.030–0.125 µg/ml (0.05 µg/mL), respectively.[15] Another collection of 81 *C. carrionii* strains revealed that itraconazole and posaconazole had the lowest MIC required to inhibit 90% of the strains tested (MIC_{90}: 0.063 µg/mL), followed by terbinafine (MIC_{90}: 0.125 µg/mL) and isavuconazole and voriconazole (MIC_{90}: 0.25 µg/mL). The highest values were recorded for caspofungin (MIC_{90}: 2 µg/mL), micafungin (MIC_{90}: 4 µg/mL), amphotericin B (MIC_{90}: 8 µg/mL), and fluconazole (MIC_{90}: 64 µg/mL).[16] A systematic review by Hellwig et al.[17] examined 35 studies evaluating the susceptibility of chromoblastomycosis agents to eight antifungal drugs and their combinations. Posaconazole, terbinafine, itraconazole, and voriconazole were, in descending order, the most effective anti-fungal drugs against chromoblastomycosis agents *in vitro*. In combination, only terbinafine plus voriconazole and itraconazole plus caspofungin showed 100% synergy for *Fonsecaea pedrosoi*, *Exophiala jeanselmei*, and *Phialophora verrucosa*, while none showed antagonism.

Given the paucity of clinical data, treatment of phaeohyphomycosis depends on the clinical syndrome and the immune status of the patient. For localised subcutaneous nodules, surgery alone may be curative. Itraconazole has excellent activity and has been used most, although voriconazole and posaconazole are increasingly being used with good results.[18] Amphotericin B is often ineffective. The duration of therapy varies but may range from 6 weeks to more than 12 months. For this patient, since the disease was disseminated and the possibility of an immune deficiency could not be ruled out, treatment with voriconazole and close monitoring of the number and size of lesions through ultrasound was continued for 21 months.

CONCLUSION

Despite a difficult diagnosis and a rare occurrence, physicians and surgeons should be aware of this infection, especially in immunocompromised patients. It can often be misdiagnosed as a carcinomatous, non-healing ulcer. Incomplete investigation of infectious lesions may delay diagnosis. Both histopathological and microbiological assessments are equally important for making a conclusive diagnosis. As antifungal therapy may delay growth of fungi that normally grow within a week, a longer incubation time may be warranted for suspected samples.

Surgery in combination with prolonged treatment with triazoles is effective for the eradication of phaeohyphomycosis.

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Cutaneous Mucormycosis of the Interscapular Region in an Immunocompetent Patient

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Abstract

Mucormycosis is an invasive fungal infection caused by opportunistic fungi of the phylum Glomeromycotan, subphylum Mucormycotina, mainly affecting individuals with immunosuppression. Cutaneous mucormycosis is the third most common clinical form of the disease preceded by pulmonary and rhinocerebral mucormycosis. The usual factors predisposing to this infection are individuals who are immunocompromised with conditions like HIV, haematological malignancies, and diabetes mellitus, but a significant proportion of patients are immunocompetent. The agents of mucormycosis are abundantly present in nature and are transmitted to the skin by direct inoculation. It may be due to needle sticks, stings, and bites by animals, motor-vehicle accidents, natural disasters, and burn injuries. The clinical presentation is non-specific, but an indurated plaque that rapidly evolves to necrosis (eschar) is a common finding. The infection can invade locally, and also penetrate into the adjacent fat, muscle, fascia, and bone, or become disseminated. It is difficult to diagnose because of the non-specific presentation of mucormycosis. Biopsy and culture should be performed. Treatment consists of multidisciplinary management, including surgical debridement, use of antifungal drugs (amphotericin B and posaconazole), and reversal of underlying risk factors, when possible. Mortality rates are significant, ranging from 4% to 10% in localised mucormycosis infection, but are lower than the other forms of the disease. The authors present a case here of a 38-year-old immunocompetent male with cutaneous mucormycosis at the interscapular region.

INTRODUCTION

Mucormycosis is an uncommon but emerging opportunistic fungal infection with high morbidity and mortality, feared by clinicians worldwide.[1] It is a potentially lethal infection caused primarily by filamentous fungus Rhizopus, Mucor, and Lichtheimia species of the fungi of the order Mucorales.[2-6] It usually affects patients with poorly controlled diabetes and individuals with immunosuppression.[7-11] Frequent clinical presentations include rhino-cerebral, pulmonary, and cutaneous forms, and less frequently, gastrointestinal, disseminated, and miscellaneous forms.[12-16]

This case report will focus on cutaneous mucormycosis.

CASE REPORT

The authors had a 38-year-old patient presented to the clinic with the chief complaint of a painful indurated ulcer measuring 2x3 cm at the interscapular region, with pus discharge for 2 days. The patient had no history of diabetes, HIV infection, or prolonged corticosteroid therapy. The patient reported no trauma and was unsure about insect bite. The patient was admitted to wards and started on cefoperazone sulbactam and tramadol. Debridement of the ulcer was undertaken on Day 1 of admission. On post-operative Day 2, the entire wound was filled with pus and black discolouration. Clinical examination of the infected area revealed a large black necrotic patch measuring 6x6 cm with a satellite lesion measuring 1.5x2.5 cm at the interscapular region (Figure 1).

On the second hospital day, debridement was repeated, and the sample was sent for microbiology and histopathology work-up. Daily dressing of the wound was undertaken with betadine (povidone–iodine) and hydrogen peroxide, and the patient was continued on medications. On histopathology exam, the findings were thick, broad, non-septate fungal hyphae suggestive of mucormycosis (Box 1).

Systemic amphotericin B was started on the day mucormycosis was confirmed. Simultaneously, daily local dressings were undertaken for the wound with povidone–iodine, hydrogen peroxide, and normal saline, along with topical amphotericin B deoxycholate.

Extensive surgical debridement was done on the fourth hospital day, and the patient was continued on amphotericin B and routine wound care (Figure 2). The patient developed fever and chills which improved with supportive care. The patient's wound showed improvement over next 3 weeks from pale granulation tissue to healthy granulation tissue (Figure 2). Split-skin grafting was performed using auto graft from the left thigh under general anaesthesia. The graft showed 100% uptake with a healthy wound (Figure 3).

The patient was continued on a single dose of amphotericin B at 1 mg/kg body weight as an infusion in 100 mL of 5% dextrose over 1–2 hours for a period of 45 days. The patient was doing well after 20 months since hospital discharge.

DISCUSSION

Mucormycosis is the common name given to several different diseases caused by fungi of the order Mucorales. Mucormycosis is an opportunistic fungal infection, usually occurring in patients who are immunocompromised but can infect healthy individuals as well. Predisposing factors for mucormycosis are uncontrolled diabetes, malignancies such as lymphoma and leukaemia, renal failure, organ transplant, long-term corticosteroid use and immunosuppressive therapy, cirrhosis, burns, protein–energy malnutrition, and AIDS. The current patient was a young male with full immunocompetency and no comorbidities.

In patients who are immunocompetent, phagocytosis of the spores prevents a fulminant course of the infection. Macrophages and neutrophils play an important role in this process. However, in individuals who are immunocompromised, lack of production and disruption of function of these immune cells lead to a rapid, progressive course of disease.

Manifestation of cutaneous mucormycosis is variable and can present gradually or as a fulminant disease leading to dissemination.[17]

This case report illustrates that the diagnosis of cutaneous mucormycosis can be difficult because initial symptoms are often non-specific and can mimic a variety of infectious skin diseases. This underlines the utmost importance of early harvesting of soft-tissue probes from the lesion site, in cases of progressive signs of infection.[18] Biopsy samples should be attained early, so treatment can be initiated. Pathological features include angioinvasion, which initiates thrombosis and infarction of the affected surrounding tissues, leading to necrosis. The presence of wide, twisted fungal hyphae within blood vessels, with necrosis of the tissues supplied by affected vessels, is diagnostic for mucormycosis. Microbiological studies can then delineate the species of fungi involved.[19]

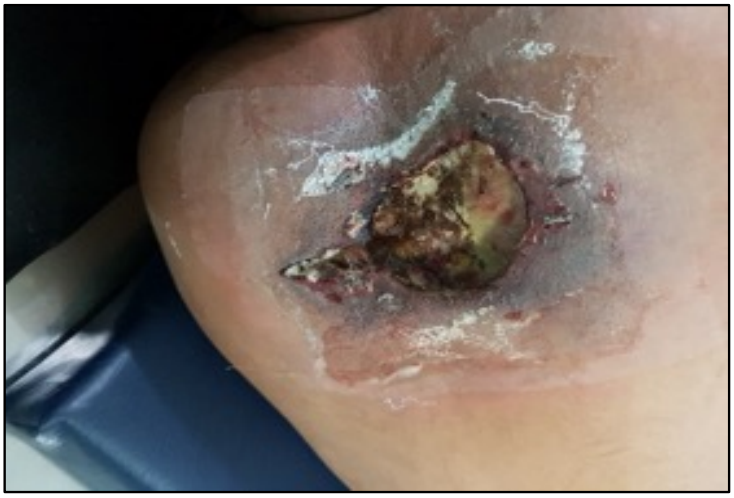

Figure 1: Clinical presentation of patient after first debridement.

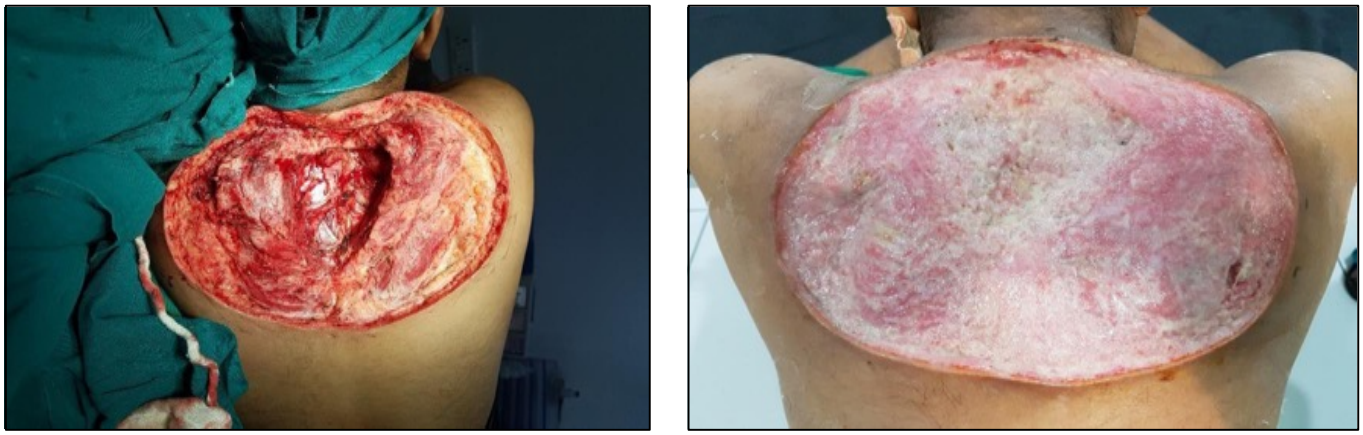

Figure 2: Final debridement (left) and healthy granulation tissue (right).

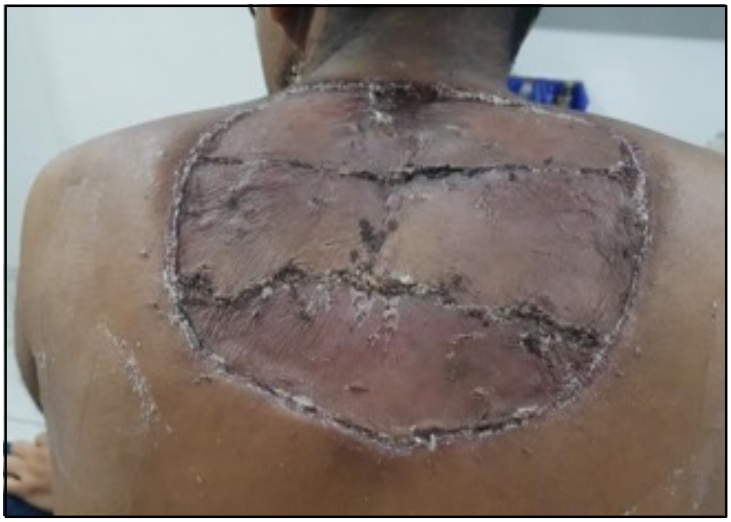

Figure 3: Post-skin grafting.

Box 1: Histopathology and culture reports.

Histopathological examination

Specimen: tissue from back for histopathological examination.

Clinical diagnosis: fungal infection in necrotic-gangrenous patch on back.

Gross description:

Received two grey–brown soft-tissue fragments, measuring in total 2.3x1.3x0.5 cm. All embedded.

Section taken:

A-1 (HE)

A-1 (PAS)

B-1 (HE)

Microscopic description:

Sections show non-viable fibromuscular and adipose connective tissue with many thick, broad, non-septate fungal hyphae, morphologically consistent with mucormycosis. There is no evidence of granulomatous pathology or malignancy in the specimen studied.

Diagnosis:

Fungal infection morphologically consistent with mucormycosis.

Correlation with clinical, radiological, and microbiological fungal culture findings is suggested.

Culture for fungus report:

Specimen:	Tissue.
KOH preparation:	Few thick, broad, aseptate ribbon like hyphae are seen.
Gram stain:	Scanty pus cells, many Gram-negative bacilli are seen.
ZN stain:	AFB not detected.
Organism:	Mucor species.
Colony count:	Profuse.
Note:	Cultural and morphological characters from growth are suggestive of Mucor species. Please correlate with history and clinical conditions of the patient.

AFB: acid-fast bacillus; HE: haematoxylin and eosin; KOH: potassium hydroxide; PAS: periodic acid-Schiff; ZN: Ziehl-Neelsen.

A multi-modal approach in the management of cutaneous mucormycosis has been demonstrated to improve overall survival. This involves reversing any risks and underlying contributing comorbidities, systemic antifungal treatment, and aggressive surgical debridement. Debridement optimises cure rates by preventing further dissemination to deeper organs and manages the extensive necrosis occurring that may not be prevented by killing the organism with antifungals. When combined with early, high-dose systemic antifungal therapy, studies have shown that mortality can be reduced to less than 10%. Due to the resistance of Mucorales, the antifungal agent of choice is typically amphotericin B at high doses. However, because of its nephrotoxicity,

renal function needs to be monitored. Novel regimens include using combination therapies (echinocandin or itraconazole) and adjunct treatments such as hyperbaric oxygen.[20]

CONCLUSIONS

The key to early detection and management of mucormycosis having a high index of suspicion for the disease. Ulcers with necrotic edges are not uncommon; however, its rapidly progressive nature should warrant suspicion of this disease. Identification of known risk factors, coupled with clinical findings and unresponsiveness to usual treatment, should prompt investigations. In this way, treatment can be initiated early to prevent a fulminant course of disease.

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