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ECCMID 2022

EDITOR'S PICK

Are Positive Urine Cultures Directly Correlated with Elevated Levels of Novel Biomarkers for Childhood Urinary Tract Infection?

INTERVIEWS

Ron Daniels and David Heymann, two leading microbiology and infectious diseases experts, spoke to EMJ about their research interests and landmark publications

Contents

"This year, the EMJ team travelled to Lisbon to attend the 32nd ECCMID in person: our first in-person congress in over 2 years, and the first ever in person ECCMID congress since launching this journal in 2020."

Editorial Board

VIEW IN FULL ←

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

EMJ aims to support healthcare professionals in continuously developing their knowledge, effectiveness, and productivity. The editorial policy is designed to encourage discussion among this peer group.

EMJ is published quarterly and comprises review articles, case reports, practice guides, theoretical discussions, and original research.

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Front cover and contents photograph: Lisbon, Portugal, home of ECCMID 2022. © Kavalenkava / AdobeStock

EMJ Welcome letter

Dear Readers,

Welcome to the 2022 issue of *EMJ Microbiology and Infectious Diseases,* bringing you key updates from this year's European Congress of Clinical Microbiology and Infectious Diseases (ECCMID).

This year, the EMJ team travelled to Lisbon to attend the 32nd ECCMID in person: our first in-person congress in over 2 years, and the first ever in-person ECCMID congress since launching this journal in 2020. ECCMID was a fantastic experience and a real privilege to attend, and we are delighted to bring you some key highlights in this issue.

With COVID-19 and antimicrobial resistance being the focal points of congress sessions, there were a lot of highly stimulating discussions of lessons learnt from the pandemic, and key strategies to combat antimicrobial resistance. Due to its perplexing nature, the emergence of hepatitis of unknown aetiology in children was also discussed in a late-breaking session. It is knowledge like this, shared at ECCMID, that can make a difference to future safeguarding against infectious disease. For example, the recent outbreak of monkeypox in non-endemic countries means that now, more than ever, information sharing and learning from past experiences will be key.

As always, in addition to the congress content, we are bringing you highly engaging reviews, research articles, and case reports. Our Editor's Pick is a narrative review discussing novel biomarkers in urinary tract infections, and other articles include a review on the pathogenesis of Zika virus and a retrospective 5-year study on infectious disease trends observed in patients in a tertiary hospital.

I would like to take this opportunity to give a big thank you to our authors, our reviewers, and of course our Editorial Board who have helped elevate the quality of the content. I hope you enjoy reading this issue, brought to you by the EMJ team.

Koutsouki

Evgenia Koutsouki, PhD.

Editor

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Foreword

Dear Colleagues,

It is a great pleasure to present the latest issue of *EMJ Microbiology and Infectious Diseases*. As with previous issues, you will find an immersive array of clinically relevant, peer-reviewed articles within these pages.

My Editor's Pick is a narrative review summarising novel biomarkers for paediatric urinary tract infections. The identification of novel biomarkers that are more accurate than conventional screening tests might help to enhance diagnosis and minimise inappropriate antibiotic treatment in children with this condition. This paper adds to the growing body of literature on biomarkers in urinary tract infections and will be a stimulus for the EMJ readership.

Furthermore, this issue features a timely review on the role of aviptadil as a therapeutic option against severe acute respiratory syndrome coronavirus 2. This drug has proved to be effective in the treatment of severe respiratory failure as a result of lung infection or injury and holds a promising place in the treatment armamentarium of COVID-19. By publishing such articles, *EMJ Microbiology and Infectious Diseases* contributes to the ongoing global effort to overcome the COVID-19 crisis.

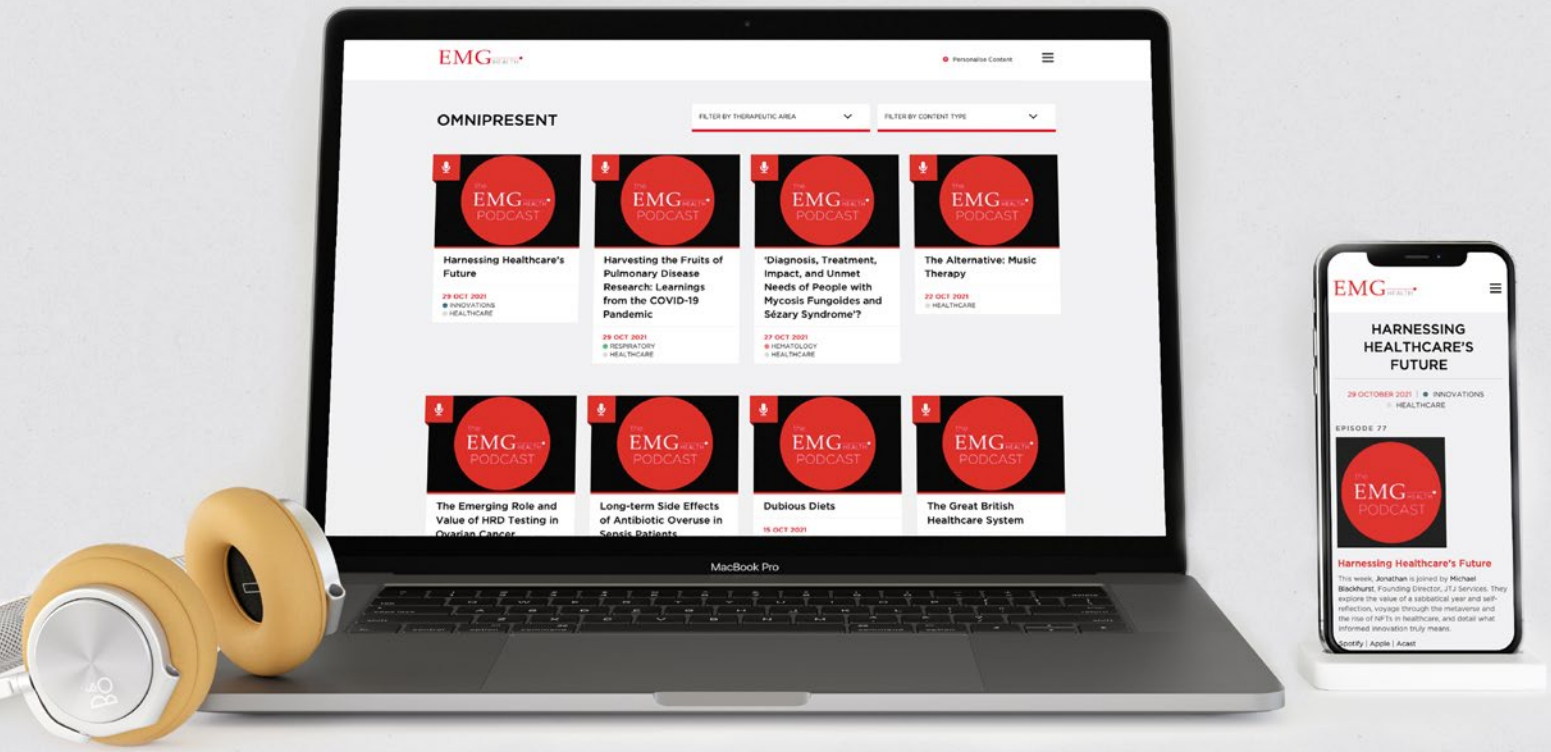
A spotlight is also shone on sepsis. Our fascinating interview with Ron Daniels, Chief Executive and Founder, UK Sepsis Trust, is not to be missed. Daniels spoke to EMJ about the Sepsis Six treatment pathway, Red Flag Sepsis, and innovations in rapid diagnostic tests for sepsis. This is complemented by an in-house infographic covering sepsis recognition tools and current approaches to management.

For those who were unable to attend this year's European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), I recommend reading the Congress Review. This features late-breaking research news from the meeting as well as a compelling feature and abstract review highlights.

I would like to congratulate all authors, interviewees, Editorial Board members, and reviewers who contributed to successfully create the 2022 issue of *EMJ Microbiology and Infectious Diseases*. I hope this journal will prove an inspiring read and a valuable resource to assist in daily practice.

Rajeshwar Reddy Kasarla

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



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Congress Review

Review of the 32nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) 2022

Location: Lisbon, Portugal

Date: 23rd–26th April 2022

Citation: EMJ Microbiol Infect Dis. 2022;3[1]:11-23. DOI/10.33590/emjmicrobiolinfectdis/22E0607. https://doi.org/10.33590/emjmicrobiolinfectdis/22E0607.

A PIONEERING hybrid format was adopted by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) for the 32nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), allowing delegates to meet onsite in Lisbon, Portugal, and also online. During the Opening Ceremony, Maurizio Sanguinetti, ESCMID President, commented: "It's singular that this congress opens in Lisbon because this gives me the opportunity to draw a parallel between the Lisbon story and the ECCMID story." The Great Lisbon earthquake of 1755 was an unprecedented catastrophe, which almost razed the capital to the ground. "We can, therefore, say that the history of Lisbon includes two major periods: one before the earthquake and the other one after the reconstruction. Similarly, we could divide the ECCMID story in two stages: before and after COVID-19," said Sanguinetti.

Combining both an in-person and virtual event experience proved to be hugely popular, as evidenced by statistics shared during the Opening Ceremony. Jacob Moran-Gilad, ECCMID Programme Director, revealed that ECCMID 2022 had already attracted 13,327 participants. "I'm sure that in the coming days, the numbers will increase," added Moran-Gilad. "We have a record number of countries," stated the ECCMID Programme Director. A total of 156 countries were represented, "so most countries in the world, which is amazing." Clearly, ECCMID continues to remain one of the world's leading clinical microbiology and infectious diseases congress.

According to Moran-Gilad, the 2022 scientific programme was notable for featuring "an amazing panel of keynote speakers." Lectures covered a broad range of topics, such as the impact of climatic change on neglected tropic diseases in lower middle-income countries, future threats from coronaviruses, clinical research in infectious endocarditis, and advances in clinical antibacterial resistance research.

Symposia also spanned across the disciplines, providing updates on HIV treatment and prevention, artificial intelligence in disease management and control, the management of bloodstream infections in immunocompromised patients, and the prediction and prognosis of *Staphylococcus aureus* pneumonia. Of particular relevance was the session entitled 'Past, Present and Future of Pandemics: Preparedness and First Defense'. This forms the basis of our compelling in-house congress feature, which considers the global spread of human pathogens and measures to contain them.

An overview of standout ESCMID press releases can be found within this issue of *EMJ Microbiology and Infectious Diseases*, including the impact of a longer interval between COVID-19 vaccine doses on antibody levels, whether a 4-week occupational therapy programme can relieve long COVID fatigue, and the identification of existing medicines that could be repurposed for gonorrhoea treatment. Each news story

featured in our independent congress review was based on research presented during ECCMID 2022.

Several major awards were presented as part of ECCMID 2022. Michele Bartoletti, IRCCS Sant'Orsola and University of Bologna, Italy, and Timothy Rawson, Imperial College London, UK, both received the ESCMID Young Investigator Award for Research in Clinical Microbiology and Infectious Diseases. Bartoletti's research focused on the epidemiology and clinical management of infections in patients with liver cirrhosis. Rawson investigated whether artificial intelligence and real-time monitoring could support optimised antimicrobial usage. Furthermore, this year's recipient of the prestigious ESCMID Award of Excellence was Bart Haagmans, Erasmus Medical Center, Rotterdam, the Netherlands. Haagmans's research focuses on the pathogenesis of viral infections, especially viruses that emerge through zoonotic transmission, such as severe acute respiratory syndrome coronavirus 2, as a basis for future interventions.

"I'll finish off with a forward look," concluded Moran-Gilad during the Opening Ceremony. Moran-Gilad believes that the future is hybrid: "No doubt, hybrid congresses are here to stay and we should work to constantly improve the format." He emphasised that, "at some point, we hope the pandemic will subside, but pandemic preparedness, health security, and public health and public policy should, and shall, remain an important topic in the programme."

Whatever the future holds, conferences such as ECCMID are crucial for the generation and exchange of scientific knowledge. With this in mind, we look forward to being part of the international clinical microbiology and infectious diseases community again, at next year's congress in Copenhagen, Denmark. Until then, read on for our key scientific insights from ECCMID 2022. ■

"Clearly, ECCMID continues to remain one of the world's leading clinical microbiology and infectious diseases congress."

ECCMID 2022 REVIEWED →

Virtual Exercise Classes Help Improve COVID-19 Symptoms

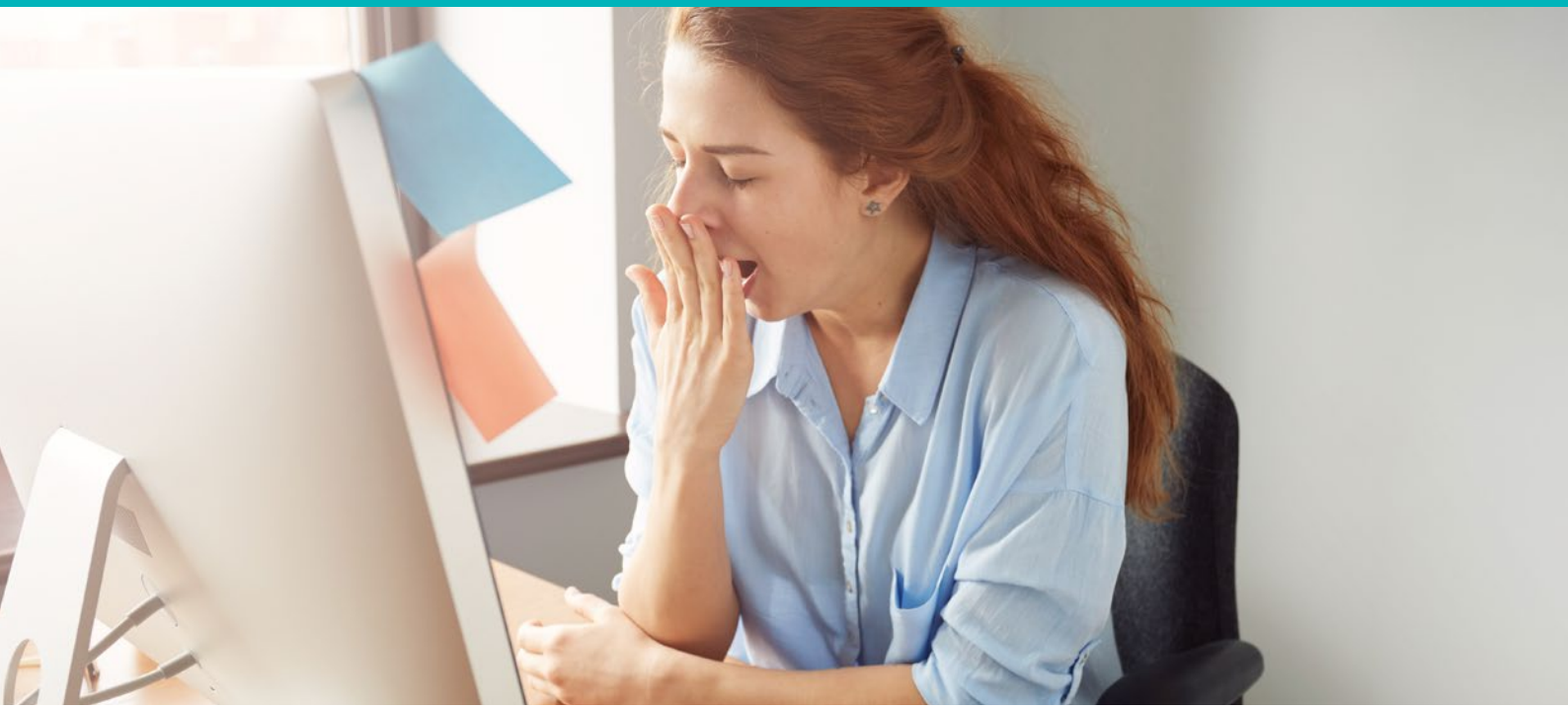
COVID-19 symptoms that persist following recovery from the initial acute infection can be improved by virtual exercise classes, a new study suggests. These symptoms, which include fatigue, breathlessness, joint pain, and chest pain, are often debilitating to patients even years following COVID-19 infection, severely impacting their quality of life and ability to carry out normal day-to-day activities. An Irish study presented at ECCMID 2022, 23rd–26th April 2022, in Lisbon, Portugal, shared the results of a virtual 6-week exercise-based recovery programme, which was designed to aid COVID-19 patients by reliving these enduring symptoms.

The programme consisted of two 50-minute virtual classes a week, for a 6-week period, involving a wide range of exercises such as squats, lunges, stretches, and other aerobic and strength-based exercises, the intensity of which gradually increased over the sessions. The study was comprised of 60 patients (42% male, average age 45 years), whose physical fitness and breathlessness, fatigue, and health-related quality of life were assessed at the start of the investigation by the 6-Minute Walk Test (6MWT), Chalder Fatigue Score (CFQ), and Short-Form-36 (SF-36) scores, respectively.

The results collected after 6 weeks showed that, out of 40 patients who completed the programme, 34% were able to walk further than at the start of the study, and 70% experienced a significant improvement in fatigue levels. Improvements were also seen in other everyday activities, such as climbing stairs and carrying groceries.

"These preliminary findings suggest a physiotherapist-delivered virtual post-COVID-19 recovery programme can improve exercise capacity, breathlessness and quality of life without exacerbating fatigue."

Physiotherapist and lead researcher of this study Kate O'Brien, St James's Hospital, Dublin, Ireland, commented on the importance of these results: "These preliminary findings suggest a physiotherapist-delivered virtual post-COVID-19 recovery programme can improve exercise capacity, breathlessness and quality of life without exacerbating fatigue." ■



Four Week Occupational Therapy Helps Fatigue in Long COVID

FATIGUE is defined as chronic tiredness and is one of the many long-term symptom's patients with long COVID are burdened by. Long COVID affects at least 10% of individuals in Ireland who have previously had a COVID-19 infection, preventing people returning to their normal lives (i.e., work). An Irish study that was shared at this year's ECCMID in Lisbon, Portugal, aimed to share results from a 4-week therapy programme.

"Seventy-two percent of patients also reported to have severe breathing difficulties and issues with memory and concentration."

The therapy took place at St James's Hospital, Dublin, Ireland, and involved a total of 53 patients, predominately female (73%), who had reported moderate-to-severe fatigue as a symptom of long COVID that was affecting their daily life. Seventy-two percent of patients also reported to have severe breathing difficulties and issues with memory and concentration. As a result of these symptoms, 74% of individuals were unable to return to work at full capacity and 58% struggled to complete day to day activities, including meal preparation, walking, and driving.

In order to help patients manage their symptoms of long COVID, particularly fatigue, the cohort were assigned three online group interventions lasting 1.5 hours over the course of 4 weeks. The purpose of these virtual interventions was to provide resources and techniques to individuals to help them manage their fatigue and 'brain fog' better.

Patients were given questionnaires to complete regarding fatigue and energy levels, quality of life, and well-being before and after the programme to assess whether the group interventions have been beneficial in helping patients deal with their fatigue.

Results from this pilot study were very promising as the preliminary analysis showed patients had significant improvement in fatigue, quality of life, and well-being. This indicates that providing patients with a variety of practical techniques could be very beneficial in improving these persistent symptoms of long COVID. Due to these positive outcomes, the study has been extended and the researchers are continuing to collect data in this area. ■

Hospitalisation and ICU Admission Among Females Who Are Pregnant with COVID-19

ACCORDING to research presented at ECCMID 2022, females who are pregnant with COVID-19 are at a higher risk of hospitalisation and severe disease than females of childbearing age who are not pregnant. Results were obtained from a time-matched cohort study conducted by Kiera Murison and colleagues from the University of Toronto, Canada.

The researchers found that females who are pregnant are only half as likely to have a severe acute respiratory syndrome coronavirus 2 infection compared with the base population. However, they are approximately five-times as likely to be admitted to hospital with COVID-19 relative to females who are not pregnant of reproductive age. In addition, females who are pregnant were more than six-times as likely to require treatment in the intensive care unit.

Subsequent analyses compared females with similar comorbidities. Overall, healthy females who are pregnant are over five-times as likely to be hospitalised compared with their healthy non-pregnant peers. Interestingly, females who are pregnant with underlying illnesses were only twice as likely to be hospitalised relative to females who are not pregnant with comorbidities.

Murison summarised the research results: "These findings suggest that in otherwise healthy women, pregnancy itself seems to be a factor that increases illness severity, while among women with comorbidities it becomes one of several factors that augment risk."

In summary, this study indicates that although females who are pregnant might be at a decreased risk of severe acute respiratory syndrome coronavirus 2 infection compared with the general population, their risk of severe illness is substantially increased following infection. Therefore, COVID-19 vaccination during pregnancy is recommended. "Our findings underscore the need for clear accurate information to reassure pregnant women and tackle concerns about COVID-19 vaccine safety," said Murison.

It is important to note that there were several limitations. This was an observational study, meaning causality could not be established. In addition, the researchers could not eliminate the possibility that unmeasured factors, such as underlying medical conditions, may have impacted the results. ■

"According to research presented at ECCMID 2022, females who are pregnant with COVID-19 are at a higher risk of hospitalisation and severe disease than females of childbearing age who are not pregnant."

Cytokine Signature in Patients with COVID-19 with Worst Prognosis

"This allowed the researchers to build a decision tree to predict those patients at risk of a negative outcome."

A DYSREGULATED cytokine storm during severe acute respiratory coronavirus 2 infection can cause an aggressive inflammatory response, leading to organ failure and death in patients with COVID-19. Research presented at ECCMID 2022 aimed to identify the most relevant cytokines driving this process, which, when assayed on admission, would allow individuals with the worst prognosis to be identified.

Emanuela Sozio, Infectious Disease Clinic, Azienda Sanitaria Universitaria Friuli Centrale, Udine, Italy, and colleagues performed a retrospective study of 415 patients hospitalised with COVID-19 between May 2020 and March 2021. Patients were classified as having mild/moderate disease or severe/critical disease. In total, 15.7% of patients died in hospital and 23.6% had a negative outcome, such as orotracheal intubation or death.

On admission, serum levels of a large panel of cytokines were measured and compared against outcomes, in combination with other blood-based biomarkers. This allowed the researchers to build a decision tree to predict those patients at risk of a negative outcome.

Individuals were initially split into two groups according to their IL-6 levels. Next, levels of IL-10, mid-regional pro-adrenomedullin, soluble IL-2 receptor α, interferon-γ-inducing protein 10, and C-reactive protein were utilised to determine whether patients were at risk of a negative outcome.

Sozio commented on the wider implications of the research findings: "It is not always possible to determine which patients with COVID-19 have the worst prognosis, especially early on. It is becoming increasingly clear, however, that the earlier we treat excessive inflammation, the more likely we are to turn it off quickly and definitively and so avoid irreversible organ damage." Sozio added: "Our work may help select patients with worse prognoses who need to be admitted to high dependency units, as well as potentially help personalise their treatment." ■

Longer Gap Between COVID-19 Vaccines Results in Nine-Times More Antibodies

VACCINES have proven to be an efficient way of controlling the COVID-19 outbreak and reducing the risk of hospitalisation. However, could the length of time between getting each dose be important regarding the efficacy of the vaccine? New research delivered at ECCMID 2022 investigated whether a longer interval could affect antibody production.

Ashley Otter, Technical Lead for SIREN serology, UK Health Security Agency, and the team measured antibody levels from blood samples from approximately 6,000 healthcare workers following Pfizer (New York City, New York, USA)/BioNTech (Mainz, Germany) COVID-19 vaccination. From this sample, 3,989 patients had their first dose 21 days earlier and 1,882 had their second dose 14 days earlier. Individuals were grouped based on previous COVID-19 infection, naïve, or no history of infection.

Findings from this analysis showed that participants with previous COVID-19 infection had 10-times higher antibody levels compared with naïve participants. Additionally, examining the intervals between vaccination doses showed that having a longer dosing interval was linked with nine-times higher antibody levels in naïve individuals.

Participants with a previous infection had no difference in antibody levels due to varying dosing intervals. Moreover, patients who had their first dose of COVID-19 vaccine after 8 months of a COVID-19 infection had seven-times higher antibody levels compared with individuals vaccinated 3 months after a COVID-19 infection. Fascinatingly, the results also revealed that female and ethnic individuals were more likely to have higher concentration of antibodies.

Otter summarised the key findings of the study: "This study shows that an interval of 10–12 weeks between vaccine dose 1 and dose 2 results in higher antibody responses compared to a 2–4-week period in participants with no previous infections. The current COVID-19 vaccination programme advises a 12-week interval between vaccinations and this study further supports this timeframe."

Finally, she added her hopes for the future: "Further research is needed to determine whether these higher antibody levels provide greater protection against COVID-19 disease and how this longer dosing interval may affect booster responses." ■

"Individuals were grouped based on previous COVID-19 infection, naïve, or no history of infection."

Are Partially Vaccinated Individuals at Lower Risk of COVID-19 Than Unvaccinated Patients?

COVID-19 has resulted in the fastest vaccination production in human history. Previously, creating and approving a vaccine could take up to 10 years; however, due to the collaborative approach taken by global pharmaceutical companies and universities, the first COVID-19 vaccine was approved for use in just 10 months. A study shared during ECCMID 2022 compared intensive care unit (ICU) admission and death between individuals partially vaccinated and individuals who were unvaccinated.

The scientists aimed to examine the risks of ICU admission and death in unvaccinated and vaccinated patients by analysing data from a Canadian database. The data was taken between January 2021 and January 2022 and included 20,064 individuals. Sixty-nine percent of this cohort were aged 50 years and over. Additionally, out of this sample, 3,353 were vaccinated and 16,711 were unvaccinated.

Methods of comparison included matching each vaccinated patient with up to five unvaccinated patients. This was done because the response to COVID-19 was constantly changing, as was the dominant variant. The team used modelling techniques and unmatched analyses to determine the risk of ICU admission and death and discover the differences in vaccine effects.

"Interestingly, the team discovered there was no significant differences in risk due to the different variants."

Results were favourable for vaccinated individuals as the findings showed that individuals who were vaccinated with one, two, or three doses had significantly less risk of being admitted to the ICU and death. Interestingly, the team discovered there was no significant differences in risk due to the different variants.

Alicia Grima, Epidemiology Student, University of Toronto, Ontario, Canada, and co-author of this study, shared her opinion on the research results: "Even with the diminished efficacy of vaccines against infection with novel variants of concerns, our findings indicate that vaccines remain a vital tool for reducing ICU admission and death from COVID-19."

Although this study highlighted the importance of COVID-19 vaccination and the reduced risk of ICU admission and death, the session added that it is still important to note that factors that were not measured (e.g., previous infection) could have affected the results in the unvaccinated group of individuals. ■

COVID-19 Death Rates Three-Times Higher Than Seasonal Influenza

A SPANISH STUDY conducted during the first wave of the COVID-19 pandemic has found that adults in hospital due to the disease were three-times as likely to die within 30 and 90 days, respectively, than those patients hospitalised for bouts of seasonal influenza. Results were shared during ECCMID 2022.

The retrospective cohort study, carried out at the Hospital del Mar, Barcelona, Spain, discovered that adults over the age of 18 years, who are hospitalised with COVID-19, have a higher risk of complications and death than patients with influenza. This is despite their younger age, and less chronic illnesses.

Also linked in the study is the association of COVID-19 with lengthier stays in hospital and intensive care than influenza. Researchers also found COVID-19 costs almost twice as much to treat than influenza, at over 21,000 EUR per patient.

"COVID-19 patients had consistently worse health outcomes."

In their study, researchers examined the medical records of 187 patients (average age: 76 years; 55% male), all admitted to hospital with seasonal influenza between 2017 and 2019. They compared these records to 187 patients hospitalised with COVID-19 between March and May 2020 (average age: 67 years; 49% male). All of the COVID-19 patients required O_2 therapy upon admission.

In both cohorts, researchers chose to enrol patients continuously until the required sample size was met. The study went on to compare clinical characteristics between patients; healthcare resource use outcomes, including duration of stay and intensive care admission; hospital costs; and rates of death.

The study discovered that patients hospitalised with influenza had more existing chronic illnesses, and challenges with daily living, than the cohort of patients with COVID-19, but were less likely to be overweight. It also found that COVID-19 was associated with a raised infection severity risk, and higher admission to intensive care. Patients with COVID-19 were also at more risk of developing complications such as blood clots, moderate-to-severe acute respiratory distress syndrome, and acute kidney injury. Patients with influenza were more likely to experience bacterial pneumonia. After taking into account age, sex, disease severity, comorbidities, the presence of pneumonia, and corticosteroid treatment, the research team concluded that COVID-19 is a much deadlier disease than influenza. Lead study author Inmaculada Lopez explained: "Our findings suggest COVID-19 is far more lethal than influenza." She added: "COVID-19 patients had consistently worse health outcomes." ■

Antibiotic Prescriptions Fall Significantly During COVID-19 Pandemic

A STUDY presented at ECCMID 2022 has found that the number of outpatient antibiotic prescriptions in Australia fell by up to 38% during the COVID-19 pandemic. In winter 2020, 38% fewer prescriptions were issued than during the same period in 2018 and 2019 (1.432 million on average per month versus 2.313 million). Twenty-three percent fewer prescriptions were issued during the summer of 2021 in comparison to the summers of 2018 and 2019 (1.347 million on average per month versus 1.817 million). Similar falls in prescription numbers were noted across the country, in states with and without lockdowns.

In this study, researchers examined rates of outpatient antibiotic prescriptions throughout Australia before and during the pandemic. A representative sample of 10% of these prescriptions issued between January 2014 and April 2021 demonstrated that before the pandemic, a clear seasonal variation existed. Before 2020, in Australia, prescriptions of antibiotics were 29% higher during the winter months (June–August). The study found that this usual seasonal variation was not replicated during the pandemic. Numbers dropped sharply in March 2020, coinciding with strict national restrictions, and remained lower than usual during the remainder of the period studied. Decreases were noted across all age groups, and the largest of these was observed in the 0–17 year cohort.

These findings mirror other studies carried out globally, which have shown similar reductions in antibiotic prescriptions to outpatients during the pandemic. Reasons for this are somewhat unclear, but multifactorial. They could include the inability or fear of seeing a clinician in person; strict lockdown measures; or other public health measures, including targeted mask wearing and culture shifts with regard to hygiene. Reductions may also be partially explained due to social distancing measures, which also reduced the spread of respiratory infections like influenza.

Lead study author Jack Skeggs, Monash Infectious Diseases, Monash Health, Clayton, Australia, commented: "Antibiotic resistance threatens many of the gains of modern medicine in increasing life expectancy and decreasing infant mortality and hugely increases peri-operative risk." He went on to stress that "reducing unnecessary use of antibiotics is the first and least costly step in preventing the development of antibiotic resistance." ■

"Reducing unnecessary use of antibiotics is the first and least costly step in preventing the development of antibiotic resistance."

Disparities in Antibiotic Prescriptions in the USA

"Antibiotic prescribing is a big issue in the USA compared with other countries, and most of this overprescribing happens in outpatient settings."

DISPARITIES in inappropriate antibiotic prescribing for older, Black, and other ethnic minorities has been observed over the past 7 years in hospital clinics and emergency departments, according to a study presented at ECCMID 2022. Approximately three-quarters and two-thirds of antibiotics prescribed to older patients over the age of 60 years and Black people, respectively, were considered improper. Furthermore, the study showed that out of the presented hospital and emergency visits, approximately 11% (around 8 billion) concluded with an antibiotic prescription.

The researchers, from the University of Texas Health Science Center, San Antonio, USA, carried out an observational study using the prescribing data collated from the Centers for Disease Control and Prevention (CDC) National Ambulatory Medical Care Survey (NAMCS), which covers over 5.7 billion adults (>18 years) and about 1.3 billion children (<18 years). The data is based on outpatient visits from 2009 to 2016, and covers all 50 USA states. The prescription of antibiotics was defined by the researchers as an antibiotic prescription per 1,000 visits, which allowed them to confirm if the antibiotic prescription was appropriate, somewhat appropriate, or inappropriate. They further evaluated the acquired data of prescriptions by race/ethnicity, age group, and gender to identify any disparities.

The findings showed that patients who were Black (122 per 1,000 visits) and Hispanic (139 per 1,000 visits) have the highest rates of prescriptions. Additionally, children had 114 prescriptions per 1,000 visits in total, while female patients had 170 antibiotic prescriptions per 1,000 visits. The researchers investigated the data to determine appropriateness and discovered that roughly two-thirds and over one-half of prescriptions to patients who are Black and Hispanic, respectively, were inappropriate. Three-quarters (74%) of antibiotic prescriptions written to patients over the age of 65 years and over half to males (58%) were considered inappropriate. The researchers stated that one of the reasons for overprescribing in minority populations is due to physicians worrying that the patients would not return for another appointment despite the possibility of an infection. However, antibiotic prescribing is a big issue in the USA compared with other countries, and most of this overprescribing happens in outpatient settings. ■

Priority Pathogen Could Be Thwarted by Repurposed Drugs

"Better treatments for gonorrhoea are urgently needed and finding new uses for old drugs could provide a rapid and relatively inexpensive solution."

GONORRHOEA is the second most common bacterial sexually transmitted infection in the UK, with 70,936 cases being reported in the UK in 2019. Presenting their findings at the ECCMID 2022 congress in Lisbon, Lilana Rodrigues, Institute of Hygiene and Tropical Medicine (IHMT), NOVA University of Lisbon, Portugal, and colleagues believe that existing drugs can be repurposed to help treat this infection.

The World Health Organization (WHO) has labelled gonorrhoea as a "priority pathogen" due to its growing resistance to antibiotics, with some strains now resistant to treatment. Gonorrhoea can lead to serious complications, including infertility in males and females as well as complications during pregnancy such as miscarriages. Rodrigues stated: "Better treatments for gonorrhoea are urgently needed and finding new uses for old drugs could provide a rapid and relatively inexpensive solution."

The researchers identified and tested 57 potential existing drugs to see which ones effectively inhibit drug efflux, a process where bacteria pump drugs out of cells. Inhibiting this process would increase the antibiotic concentration, thereby making the bacteria more susceptible to treatment. Using computer modelling, the researchers were able to identify 30 potential 'weak spots' that these drugs could exploit.

This study saw six drugs that were promising: amlodipine, which is used to treat blood pressure; doxorubicin, a drug used in chemotherapy; and atovaquone, which can be found in some anti-malarial tablets. Other drugs that showed promise were acetazolamide, dequalinium, and clomipramine. The researchers found that some antibiotics for gonorrhoea treatment were four-times more effective when used with doxorubicin, atovaquone, and acetazolamide than alone.

Repurposing existing drugs is an attractive prospect as there is already research and clinical trials around their use. It also reduces the risk of drug development and costs. Rodrigues stated that this research could lead to further research into new treatments for gonorrhoea. ■

Congress Feature

Past, Present, and Future of Pandemics: Preparedness and First Defences

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As expected, debate and expert discussion at the 32nd European Congress of Clinical Microbiology and Infectious Disease (ECCMID) in April 2022 was set to the backdrop of COVID-19, focusing on the lessons learnt throughout the pandemic and looking to mitigating risks of future pandemics. In a session on the third day of ECCMID 2022, specialists took a holistic view, presenting evidence and testimony on past pandemics alongside a look to the future, emphasising the value of pandemic preparedness and first-line defences. Nicholas Grassly, Vaccine Epidemiology Research Group (VERG), Department of Infectious Disease Epidemiology, Imperial College London, UK, drew parallels between the polio pandemic of the past and COVID-19 in regard to the impact on populations and response of the scientific community. Nicola Petrosillo, National Institute for Infectious Diseases (INMI) Lazzaro Spallanzani, Rome, Italy, discussed the impact of COVID-19 on influenza prevalence, highlighting challenges arising from co-infections with two increasingly endemic viruses.

THE FUTURE AND PAST OF VIRAL POLIOMYELITIS

Grassly opened his presentation by providing a historical overview of the poliovirus. The virus, which evolved thousands of years ago, was endemic within the population. However, improving sanitation and hygiene were helping its gradual disappearance. However, large epidemics grew in the late 1940s and early 1950s to near pandemic levels. Drawing parallels with the COVID-19 pandemic, Grassly showed images of hospitals filled with children attached to iron lungs, the mechanical ventilation method of the time. Further salient parallels between COVID-19 and polio were noted, with Grassly highlighting the scope and level of response from the scientific community to polio. The largest placebo-controlled double-blind trial conducted in an open population at that time investigated passive

"Phase I and II trials demonstrated safety and immunogenicity resulting in the novel serotype 2 OPV vaccine, becoming the first ever vaccine to be expedited through the WHO emergency use listing."

immunisation using γ-globulin in approximately 50,000 children in 1952. This resulted in an immense public response, with parents bringing their children to clinics repeatedly to ensure their child was on the active agent and not the placebo, speaking to the atmosphere of anxiety and panic.

Jonas Salk and his team, Grassly continued, were the first to develop an effective vaccine based on the chemical inactivation of wild-type polioviruses, known as the inactivated polio vaccine. Viral poliomyelitis only has three poliovirus serotypes as it does not mutate and produce variants, which has been seen with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This meant that inactivation of the three serotypes provided a viable vaccine strategy. This was followed by further innovation with Albert Sabin's live attenuated oral polio vaccine (OPV), which was approved in 1963. The intestinal mucosal response paired with the systemic response and seroconversion the OPV resulted in increased viral replication and a stronger immune response. The efficacy and ease of administration of the OPV saw a global rollout, with a huge positive impact on case burden and deaths in the late 1950s. However, the efficacy of seroconversion in the gut caused by OPV was inhibited in children from low-income countries, which created ongoing challenges for the polio eradication programme.

The success of the polio vaccine programmes led to the 1998 World Health Assembly (WHA) committing the World Health Organization (WHO) to the goal of global eradication of poliomyelitis by 2000. The programme has seen significant victories. The last cases of wild-type poliovirus serotypes 2 and 3 were seen in 1999 and 2012, respectively. However, wild-type 1 persists and, since 2014, the risk of international spread of poliovirus has been considered a public health emergency of international concern. Currently, the programme costs approximately 1 billion USD a year and encompasses the collaboration of multiple non-governmental organisations. Despite the huge decrease in polio distribution, the disease remains in parts of the world where vaccine programmes face continuous challenges. At the end of 2021, wild-type polio was only present in Pakistan and Afghanistan, where five cases were reported. Grassly highlighted that each case of polio virus represents many infections as the rate of paralysis to infections is one in 700.

Despite being enormously successful with the polio vaccines initially, there is still viral persistence within populations. Attenuated viruses from the OPV, replicating within mucosal surfaces in the gut, can precipitate genetic diversity and mutation at attenuation sites. In most cases, the subsequent immune response overwhelms and destroys the mutated virus; however, vaccine-derived polioviruses can, rarely, survive with transmissibility and virulence, leading to circulating vaccine-derived polio outbreaks. This novel threat led to countries switching from trivalent to bivalent OPVs, with a push from governing bodies to include at least one dose of inactivated polio vaccine in vaccination programmes in a concerted effort to avoid OPV-generated viral mutation. However, outbreaks occurred, centring in Nigeria and Somalia from 2016 to 2019. Furthermore, the subsequent reduction in global immunity

following the withdrawal of OPV has resulted in the spread of vaccine-derived polio virus infections.

Further innovation in polio vaccine technology has focused on more stable oral vaccines, following a better understanding of the genetic bases of attenuation. Novel serotype 2 OPV was developed with additional stabilisation of mutations in the 5′ untranslated region. Phase I and II trials demonstrated safety and immunogenicity resulting in the novel serotype 2 OPV vaccine, becoming the first ever vaccine to be expedited through the WHO emergency use listing. This is the same type of emergency use listing that we have seen applied to numerous COVID-19 vaccines over the past 2 years. Over 200 million doses have since been delivered, with safety, impact, and efficacy monitoring ongoing.

In his concluding remarks, Grassly explained that at present, wild-type viruses are restricted to Afghanistan and Pakistan, with a recent spread to Malawi, where a case of paralysis occurred in 2021. Vaccine-derived viruses now constitute a much larger proportion of infections and are spread over a considerably wider area of sub-Saharan Africa and South Asia.

Contemporary research largely focuses on increasing epidemiologists' ability to track the spread of polio. The current process of obtaining a sequenced sample result following the onset of paralysis takes approximately 6 weeks, resulting in a 6-week delay before a vaccine response can be launched in a community; however, direct detection through nested PCR and nanopore sequencing can generate a viral protein 1 sequence in 2–3 days. This research, which has been pioneered by Grassly's team, has so far demonstrated improved cost efficiencies with non-inferior specificity when compared with current methods. Short-term implementation within the Democratic Republic of Congo detected four new outbreaks 3–5 weeks before current standard testing regimes.

The evidence presented by Grassly conveys the value of constant innovation and adequate investment in response to pandemic threats. The initial vaccine race was necessary to prevent the global prevalence of polio; however, new research on optimum vaccine programmes and efficient testing methods has been continuously required to compete in the viral arms race and mediate the pandemic threat.

INFLUENZA AND THE COVID-19 PANDEMIC

Presenting his session about the impact of COVID-19 on the spread of influenza, Petrosillo highlighted the value of preventative measures and the necessity for future pandemic preparedness. With similar initial clinical presentations to influenza, COVID-19 is associated with higher incidence of fever, shortness of breath, and diarrhoea. Laboratory results demonstrate that patients with COVID-19 tend to have lower white cell counts, with different cellular expression to patients with influenza.

Petrosillo then focused on the changes to influenza circulation that occurred throughout 2020 and 2021, when the COVID-19 pandemic was at its height. He quoted hospital data that demonstrated a huge overall decrease in cumulative hospital visits per 100,000 of the population for influenza from the years 2019–2020 to 2020–2021 in the USA. Notably, he highlighted the slight increase observed from 2021 to March 2022 as COVID-19 cases declined. Furthermore, the percentage of positive influenza test results reported by clinical labs in the USA dramatically decreased from 2019 to 2020, with the cumulative rate of laboratory confirmed influenza hospitalisations extremely low compared with the previous season. Petrosillo summarised that, as SARS-CoV-2 detections increase, the percentage of influenza positive tests decrease, which is underlined by the current increase of flu seen across Europe. Petrosillo

"Petrosillo further highlighted the value of the 'interpandemic period' we are currently experiencing for strengthening existing systems, allocating resources, and preparing surge capacity plans."

explained that the initial COVID-19 interventions had wide reaching impacts. Lockdowns, travel restrictions, closing schools, and increased emphasis of hygiene did not only decrease the circulation of SARS-CoV-2, but also caused an overarching reduction in viral transmission throughout the pandemic. Comparing mortality in patients with influenza, Petrosillo referenced a study of 6,965 patients with SARS-CoV-2 that found viral co-infection in 583 (8.4%) of patients. Co-infection with influenza virus was associated with increased risk for requiring mechanical ventilation (odds ratio: 4.14; 95% confidence interval: 2.00–8.49). Furthermore, co-infection was significantly associated with an increased risk of death.

The risk pandemics present in morbidity and mortality underline the necessity for adequate preparedness. Influenza pandemics, whether mild, moderate, or severe, affect large proportions of the population and require a multisectoral response over several months or years. Pandemic responses should encompass multiple levels, from regional to national and international scales. Petrosillo highlighted the value of pandemic planning being flexible, adaptable, and ever evolving, stating how nations should consider plans as living documents that are considerate of changes to legislation, new research, and rapidly transforming situations. This was made evident throughout the COVID-19 pandemic, where data, restrictions, and global prevalence were constantly changing. Petrosillo further highlighted the value of the 'interpandemic period' we are currently experiencing for strengthening existing systems, allocating resources, and preparing surge capacity plans.

Referring to the impact of the COVID-19 pandemic on the spread, mortality risk, and even lineages of circulation of influenza, Petrosillo emphasised the necessity for pandemic preparedness and concluded by explaining that, as many countries decrease use of their preventative and restrictive measures, we can expect to see endemic circulation of COVID-19, increasing the risk of co-infection with influenza and future viruses.

CONCLUDING COMMENTS

The lessons learnt from past and present pandemics provide a road map for methods to optimise pandemic preparedness in the future. Both speakers highlighted the value of rapid and large-scale scientific responses and the development of efficient, yet effective, vaccines within flexible governance structures that allow for expedited approval, with Grassly hailing protocols implemented for both polio and COVID-19. Looking to the future, the experts acknowledged the value of preparedness and plans that are flexible, constantly evolving, and informed by up-to-date information. In the shadow of COVID-19, pandemic preparedness has never been more relevant and, as stated by Petrosillo, the contemporary interpandemic period will determine the future risk posed to the global community.

Abstract Highlights

The following highlights spotlight several fascinating and timely abstracts presented at the 32nd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), covering topics such as COVID-19, antimicrobial stewardship, and antibiotic resistance.

Double Antibody Treatment Efficacy in Patients with COVID-19

SEVERE acute respiratory syndrome coronavirus 2 resulted in scientists all around the world collaborating to find a treatment against the deadly virus in an emergency health crisis. Vaccines were developed faster than ever before, as well as antibody treatments, which have the potential to not only protect a person from an infection but also treat the infection.

Last year, two combinations of monoclonal antibodies were approved to treat patients at high-risk of developing severe COVID-19. In an abstract presented at ECCMID 2022, findings revealed the efficacy of double antibody treatment, particularly in patients receiving bamlanivimab/etesevimab or casirivimab/imdevimab.

The study involved 97 patients during the period of March–September 2021. The cohort was predominately male (56%), had a median age of 69 years, and received dual monoclonal antibody therapy for COVID-19. Patients underwent nasopharyngeal swabs at diagnosis and 7 days after receiving monoclonal antibody therapy, as well as sequencing of the virus for all positive COVID-19 samples.

Out of this sample, 49.5% of patients were undergoing immunosuppressive treatment, 13.0% had chronic respiratory insufficiency, and 12.0% were receiving chemotherapy. Unfortunately, 10 of these patients passed away due to COVID-19 and three passed away due to unrelated reasons.

Promisingly, results showed 64% of patients did not require O_2 treatment and only 36% required low-flow O_2. The viral decay was reported to be similar in patients taking bamlanivimab/etesevimab or casirivimab/imdevimab. Moreover, there were no adverse events reported in patients receiving dual monoclonal antibody treatment, demonstrating that this treatment is safe as well as efficacious.

The authors summarised their findings by emphasising the safety and efficacy of combining monoclonal antibodies for COVID-19 treatment. They also acknowledged how the viral sequencing showed there were different spike mutations in patients, especially in those with the α variant. This discovery could help with creating more specific treatments in the future. ■

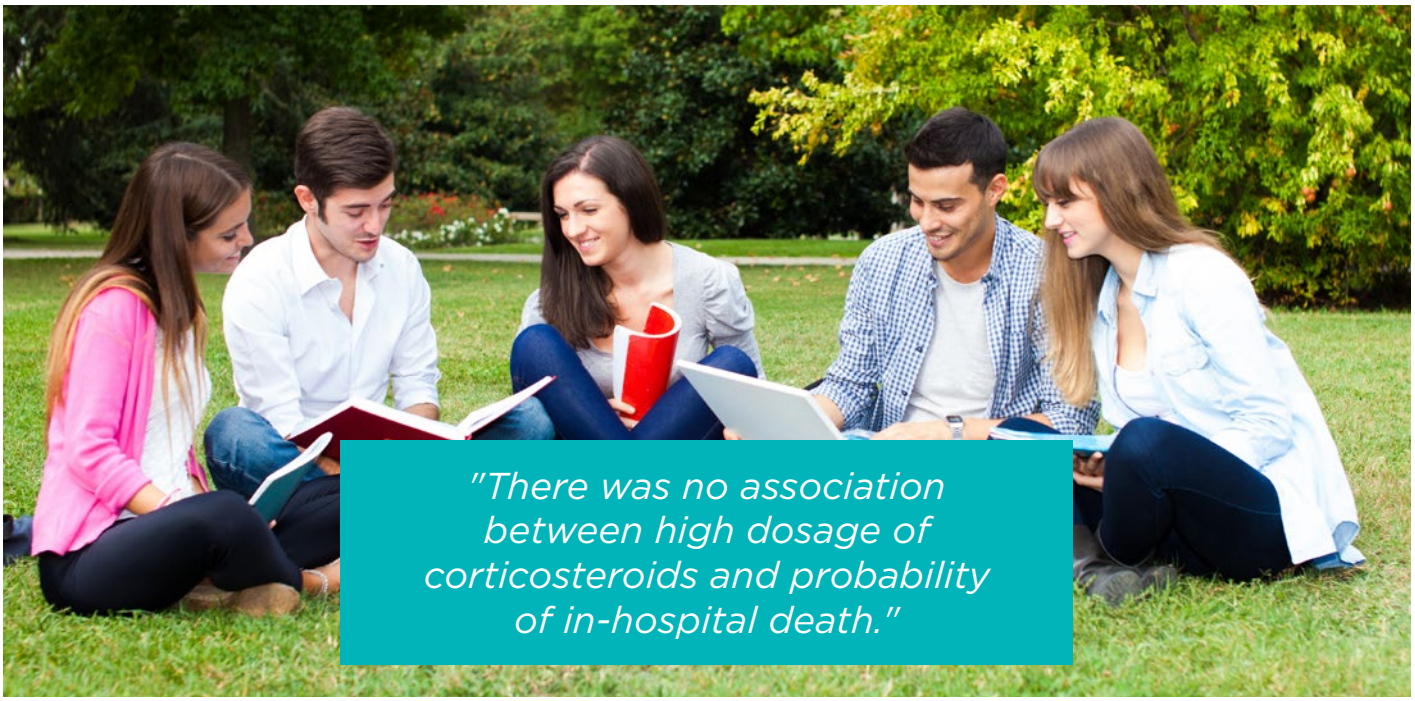
Is The Use of Corticosteroids Beneficial or Harmful to Patients with COVID-19?

ADVANTAGES and disadvantages of the effect of corticosteroids on in-hospital mortality in patients with COVID-19 who were admitted to Spanish intensive care units (ICU) were reported by a multicentre, observational cohort study presented at the 32nd ECCMID. Although there is some evidence that corticosteroids might be beneficial in severe COVID-19 cases, there is scarcely any data on certain subgroups of patients diagnosed with COVID-19.

The study included patients with confirmed severe acute respiratory syndrome coronavirus 2 infection who were admitted to 55 ICUs in Spain between February 2020 and October 2021. The included participants had been in the ICU for 2 or more days and had not received prior steroid treatment. The researchers collated demographics, clinical data, corticosteroids treatment data, complications, and outcomes.

During the period of admission in the hospital, 2,877 out of the 3,438 patients in the study (84%) had received systemic corticosteroids. The frequency of in-hospital deaths was unremarkable between those who received corticosteroids treatment (28%) and those who did not receive the treatment (31%). Following a modification of the confounding variables, it was clear that the use of corticosteroids was protective (hazard ratio: 0.66 [0.54–0.82]; p<0.001). Additionally, in the subgroup of patients aged ≥60 years, in those with higher severity, and in those with high inflammatory response (C-reactive protein: ≥150 mg/L; lymphocyte count: <0.724x10^9 cells/L), a beneficial response was reported. There was no association between high dosage of corticosteroids and probability of in-hospital death. It was observed that early administration (<7 days) was associated with higher likelihood of mortality (hazard ratio: 1.27 [1.07–1.50]; p=0.006). Finally, the use of corticosteroids was linked to a greater risk of developing nosocomial bacterial pneumonia, hyperglycaemia, and haemorrhage in the general population.

In conclusion, the findings demonstrated that regardless of the general beneficial effect, a few subgroups of patients, such as young patients, patients with a reduced inflammatory response, and patients with a less severe COVID-19 severity, will not exhibit the same benefit corticosteroids treatment. ■



"There was no association between high dosage of corticosteroids and probability of in-hospital death."

Antimicrobial Stewardship in Children Undergoing Surgery

SIMPLE antimicrobial stewardship (AS) intervention based on guidelines provision and education can lead to an increase in the appropriateness of surgical antimicrobial prophylaxis (SAP) timing and duration. In an abstract presented at ECCMID 2022, researchers explored the efficacy of AS intervention in targeting SAP in children at a 1,200-bed paediatric department in Northern Italy.

This pre- and post-intervention retrospective study assessed the appropriateness of SAP before and after an AS educational programme. The appropriateness was evaluated by protocol adherence in indication, timing, dose, and duration. The differences across the intervention groups were measured by the chi-squared test. Data were extracted from medical records of children who had cardiac, neuro-, and orthopaedic surgery.

There were 236 patients in the pre- and 203 in the post-intervention phases (N=439). In total, 372 interventions (84.7%) needed antibiotic administration before surgery. SAP was administered to most cases in both study phases (91.5% versus 91.1%); there were no significant changes in the type of surgical interventions. The rate of patients who received any SAP dose after surgery reduced (from 85.2% to 55.7%; $p<0.001$), and SAP timing was deemed more appropriate after the AS intervention (from 49.1% to 81.1%; $p<0.001$).

While patient-related risk factors for surgical site infections (SSI) increased (23.3% versus 45.3%; $p<0.001$), the rate of SSIs remained stable across both phases (3.8% pre-AS versus 4.4% post-AS; $p=0.740$). However, there was a reduction in the length of hospital stay by 3.2 days ($p=0.04$) after AS implementation.

The researchers also found that there were no significant changes in the use of antibiotic combinations (5.1% versus 8.6%; $p>0.05$).

Although there was a significant increase in patient-related risk factors for SSIs after AS intervention, SSI rates were in fact stable. This study shows that AS intervention that is based on education and the provision of guidelines can increase the appropriateness of SAP usage with regard to timing and duration, resulting in a decrease in length of hospital stay. ■

Antibiotics Prescribed Inappropriately to Children in Low- and Middle-Income Countries

"In low- and middle-income countries, data are often unavailable, particularly as the majority of the population is treated outside of medical institutions."

ANTIBIOTIC resistance is rising to dangerous levels globally, particularly within low- and middle-income countries. Often, antibiotics that are required as a treatment for resistant infections are unaffordable. Low- and middle-income countries are also faced with high levels of bacterial disease, particularly amongst the child population.

One significant factor in antibiotic resistance is the inappropriate prescription of antibiotics. In low- and middle-income countries, data are often unavailable, particularly as the majority of the population is treated outside of medical institutions. A study aimed to discover the pattern of inappropriate antibiotic prescriptions in children in Madagascar, Senegal, and Cambodia.

Data from a prospective, multicentric, community-based mother-to-child cohort (BIRDY, 2012–2018) recorded all infectious episodes in babies up to the age of 2 years. Results were taken from urban and rural areas in these three countries, including 3,710 children in the cohort. Every case of infection, with symptoms, diagnosis, and antibiotics prescribed, were examined. The study defined unnecessary prescriptions based on the diagnosis of probable bacterial infection.

In the study population, 11,762 consultations took place, 3,448 (29%) of which concluded with an antibiotic prescription. Thirty-six percent of all prescriptions consisted of amoxicillin, and 20.1% were cephalosporins. The study discovered that 57.2%, 15.5%, and 57.0% of prescriptions in Senegal, Cambodia, and Madagascar, respectively, were inappropriate. The most common disease for antibiotic prescription was bronchiolitis (43.9%), followed by gastroenteritis (35.5%), and rhinopharyngitis (20.0%).

The study also found that babies older than 3 months, with a diagnosis that had a higher severity score, increased the risk of inappropriate antibiotic prescription in all three countries. Children living in urban areas were less at risk of receiving inappropriate prescriptions.

The conclusion of the study demonstrated that due to a noteworthy percentage of inappropriate prescriptions across Madagascar, Senegal, and Cambodia, it is pivotal to create reliable local programmes that could optimise prescriptions of appropriate antibiotics on a community level. ■

Ceftazidime-Avibactam plus Aztreonam for the Treatment of Metallo-β-Lactamase-Producing Gram-Negative Bacterial Infections

IN AN abstract presentation session presented at the 32nd ECCMID, researchers from the Hospital Universitari Vall D'Hebron, Barcelona, Spain, shared the findings of their novel study investigating the optimal treatment of metallo-β-lactamase-producing Gram-negative bacteria (MBL-GNB). Currently optimal treatment of this category of bacteria is not well defined. Ceftazidime-avibactam plus aztreonam (CAZ-AVI/ATM) has previously been used to treat some patients, and the aim of the present study was to evaluate the effectiveness and safety of using CAZ-AVI/ATM to treat MBL-GNB infections.

The retrospective study analysed patients who were treated with CZ-AVI/ATM for MBL-GNB infections between November 2018 and June 2021. Twenty-three patients were included, with a median age of 64 years. The primary outcomes assessed were clinical failure at Day 14 and 30-day mortality. Synergy was evaluated through gradient strip test direct overlay using 90° angle methods.

The patient cohort represented a variety of GNB infections. Eleven patients were immunosuppressed (47%); seven had soft tissue infection (30%); six with lower tract respiratory infection (26%); three with bloodstream infections (13%); three with intra-abdominal (13%); two with occult bacteraemia (9%); one with osteomyelitis (4%); and one with urinary tract infection (4%).

Synergy tests of the combination between CAZ-AVI and ATM were performed, showing synergistic activity in 11 isolates and no synergism in one. Results of the investigation demonstrated that the median duration of the treatment was 15 days, two patients presented with clinical failure at Day 14 (9%) and three died within the first 30 days (13%). Furthermore, three patients presented with adverse effects: two with diarrhoea and one with encephalopathy.

The authors of the study concluded that in the cohort of GMB infections, CAZ-AVI-ATM was a safe and effective therapy. They summarised that this treatment should, therefore, be considered for treating MBL-GNB infections in the future. ■

Acute Bacterial Skin and Skin Structure Infections: Advantages and Disadvantages of Early Discharge and Outpatient Parenteral Antibiotic Therapy

Interviewees: Michael Wilke[1,2]

1. Inspiring-health GmbH, Waldmeisterstrasse, Munich, Germany
2. Medical School Hamburg, Am Kaiserkai, Germany

Disclosure: Wilke is Professor for Hospital Management at Medical School Hamburg as well as shareholder; is managing partner of Inspiring-health GmbH, which received a research grant from Correvio GmbH (now Advanz Pharma) to conduct health-economic analyses on dalbavancin; he is a shareholder of Pfizer; and has received honoraria for lectures and research grants unrelated to this research from Orion Pharma, Pfizer, Shionogi, Abbott, Accelerate, and bioMérieux.

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Summary

Acute bacterial skin and skin structure infections (ABSSSI) can require long periods of antibiotic therapy. If an ABSSSI is treated while a person is in hospital, this may lead to an extended hospital stay of days or even weeks, even if the patient is in a stable enough medical condition to be discharged to their home environment. As such, inpatient treatment of an ABSSSI can incur high costs for the hospital and tie up beds that could be used for other patients. Michael Wilke from Inspiring-health GmbH, Munich, Germany, and the Medical School Hamburg, Germany, discussed with the EMJ how early discharge may be viable for patients who are medically stable and can either return to the hospital for daily treatment with intravenous (IV) infusions or are able to, reliably, take oral antibiotics. Also available are the long-acting antibiotics oritavancin and dalbavancin. The latter is administered only once via IV infusion. While the cost per dose of dalbavancin is more expensive than most oral or daily/twice daily IV antibiotic regimens, its use can facilitate early discharge, leading to reduced hospital stays and offset cost savings. Due to the administration schedule, the use of long-acting antibiotics circumvents issues with daily medication adherence.

INTRODUCTION

ABSSSI includes cellulitis/erysipelas, major cutaneous abscesses, and wound infections.[1] These are most often caused by Gram-positive *Staphylococcus aureus* (including methicillin-resistant *S. aureus* [MRSA]) and *Streptococci*, with fewer ABSSSI due to Gram-negative bacteria.[1] In the inpatient population, ABSSSI presents a large healthcare burden, including greater resource use and extended hospital stays.[2]

Wilke has extensive clinical and research experience in assessing and treating ABSSSI and analysing the health economics associated with such. Here, he discusses with EMJ the medical, quality of life, and economic issues associated with patients remaining in hospital for ABSSSI treatment; how those eligible for early discharge can be best identified; and the potential advantages of the long-acting antibiotic dalbavancin (marketed as Xydalba in Europe and Dalvance in the USA [Allergan Pharmaceuticals International Ltd, Dublin, Ireland]) within these realms.

CHALLENGES WITH PATIENTS REMAINING IN HOSPITAL TO TREAT AN ACUTE BACTERIAL SKIN AND SKIN STRUCTURE INFECTION

A number of issues can arise when a person with an ABSSSI is kept in hospital only for the treatment of such a condition, discussed Wilke. The first is that the nature of the infection, deep in the skin or soft tissue, can mean antibiotic therapy needs to be administered for a number of weeks. Another is that these patients may have comorbidities such as diabetes and bad microcirculation that are associated with a higher rate of ABSSSI.[3] A deep ABSSSI in such a patient means that they may be complex to treat with regard to antibiotic efficacy and safety. For instance, dose adjustment may be needed for antibiotics due to potential drug interactions with antidiabetic agents.[3]

A further problem is financial. In many countries, Wilke pointed out, payments for treatments and conditions are limited by health insurance companies covering only a set number of days, treatments, or amount of cost per condition. This means there can be instances where a person is staying in a hospital but costs are not reimbursed, incurring a loss of finances for the hospital.[4]

Also problematic, according to Wilke, is that in hospital, the patient is more at risk of picking up another infection and of passing on the infection they have, if it is transmissible.[5] Lastly, most patients do not like staying in hospital and would prefer to be in their home environment.

CHALLENGES WITH TREATING METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

MRSA can be a particularly challenging form of ABSSSI and it is vital when a patient presents with an ABSSSI that MRSA is considered, especially when it is purulent. This is carried out by careful consideration of signs and symptoms combined with specimen culture and susceptibility testing.[6] According to Wilke, the first challenge is that MRSA can survive for long periods on surfaces,[5] leading to a risk that it is transferred from a patient to the hospital environment and back to another patient. There is also the potential that the patient can carry MRSA out of hospital and into communal living settings, such as a nursing home.[7]

The next challenge, Wilke pointed out, is that there is a very limited armamentarium of drugs with proven efficacy against MRSA. Of these, all but two of them (IV dalbavancin and oral linezolid) must be administered via daily or twice daily IV infusion, and some, like vancomycin, necessitate therapeutic blood monitoring to ensure drug levels stay within the therapeutic range.[8]

IDENTIFYING PATIENTS WHO COULD BE SENT HOME FROM HOSPITAL WITH ANTIBIOTIC TREATMENT

According to Wilke, "one in three patients with an ABSSSI could be eligible for earlier discharge as a rule of thumb." Indeed, a multicentre, multicountry study of hospital inpatients with MRSA found that 37.9% could potentially be eligible for early discharge if eligibility criteria regarding infection and clinical stability were met.[9]

Wilke discussed how he and his colleagues have developed an algorithm that can be used by healthcare professionals at the bedside to assess if a person may potentially be discharged while still being treated for an ABSSSI. This algorithm can help point to an early discharge if no infection parameters are present, for example, laboratory detection of C-reactive protein, and/or if the infection is stable or slightly declining; the patient is clinically stable; there is no fever;

the patient is not in intensive care; they have not had surgery in the last 2 days or have any planned for the foreseeable future; wound dressings, if present, could be changed in the home setting; the patient does not have issues in taking oral medication (where applicable); and, in a healthcare professional's opinion, the patient would adhere to their medication regimen.

ECONOMIC AND PATIENT QUALITY OF LIFE IMPACTS OF EARLIER HOSPITAL DISCHARGE

Being able to treat an ABSSSI in the home setting has been shown to reduce healthcare-associated costs and increase patient quality of life.[2,10] Wilke discussed how this raises the probability that a patient can be discharged within the insurance-covered length of stay for their medical condition (in countries where this applies), so that costs are at least covered, or losses are minimalised.

Also, he continued, "if a patient is discharged earlier from the hospital, the beds are free to treat a new patient." This cuts down on waiting times and, in economic terms, means more revenue can be generated per patient. Additionally, most patients, Wilke discussed, "do not appreciate being in hospital, where there are people in the room and there is no privacy." Most, he continued, "are happier in their home environment. You can organise your day as you want, not like the hospital staff wants to. In hospital, you can't just go out for a walk."

If a patient is discharged and needs to come back into the hospital for outpatient parenteral antibiotic therapy (OPAT), they are at least, Wilke highlighted, "back in life again. For instance, they can go to a restaurant and meet their children."

POTENTIAL CHALLENGES ASSOCIATED WITH DISCHARGING A PATIENT RECEIVING ANTIBIOTIC THERAPY

In many countries, treatment can be carried out via an OPAT programme where the patient attends an outpatient clinic daily or twice daily for IV antibiotics. However, while this therapy may be advantageous for people who live locally, another issue, explained Wilke, is that "if the patient does not live nearby, they might have to travel 100 km on a daily basis, which might counter the effect of being at home again." Treatment-related complications associated with IV antibiotic administration may include venous access issues, drug-related side effects, skin rash, and nephrotoxicity. If detected in the OPAT setting, such issues may lead to rehospitalisation.[10]

Although oral drug therapy may be the solution when the infection is amenable to such and a patient can take drugs orally, a challenge here, Wilke discussed, is that "current oral drug therapy with linezolid for MRSA has a limit of 28 days but some patients need treatment longer than this, so then they have to switch to another drug and go back into IV treatment."

One challenge associated with discharging a patient while they are still being treated for an infection is, according to Wilke, that "you have to track the patient and make sure that your anti-infective regimen is perpetuated." Indeed, compliance with taking a course of antibiotics is an ongoing problem in healthcare.[11,12] "Many won't take them at all after discharge," he explained and discussed how even studies using smart pill bottles with electronic sensors combined with self-report questionnaires, so the patient knows they are being monitored, show non-adherence to a drug regimen.[12]

TREATMENT WITH DALBAVANCIN

Dalbavancin is a long-acting lipoglycopeptide antibiotic used for Gram-positive bacteria such as *Streptococci* and MRSA.[13-16] With a terminal half-life of 15.5 days, the administration is a single 1,500 mg dose or a 1,000 mg dose on Day 0 and 500 mg on Day 7, administered as a 30-minute IV infusion.[15,16]

According to Wilke, dalbavancin "has advantages and a disadvantage. One advantage is that you can administer it then send the patient home as it's so long-acting." This, he explained, means that a single infusion can be given on the morning of discharge. Another advantage Wilke highlighted is that "the long half-time of dalbavancin can avoid all issues of adherence and compliance when taking antibiotics." A third advantage is that dalbavancin has a generally favourable side effect profile.[13-16]

Dalbavancin is, though, very expensive per dose. However, Wilke pointed out that while a drug such as vancomycin is much cheaper, the costs of having to remain in hospital while being treated are also very high. With vancomycin, therapeutic drug monitoring is also needed, adding to the costs that include healthcare staff and general hospital care.

A case data analysis by Wilke and colleagues found that administration of a single dose of dalbavancin in patients with an ABSSSI had the potential to reduce the length of hospital stay by 6.45 days and healthcare costs by 2,865 EUR.[4]

CONCLUSION

Treatment of ABSSSI can include many weeks of antibiotic therapy, which may mean a person has to remain in hospital even when otherwise fit for discharge. The advent of OPAT or home therapy means that eligible patients can be discharged, leading to large savings and freeing up hospital beds. However, typical IV therapy necessitates a once or twice daily return to the hospital for drug administration if oral medication is not indicated. The use of dalbavancin, though expensive, can mean a patient can be discharged from the hospital early and is advantageous in the realm of therapy adherence.

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ADV/DAL/PM/0094

Factors Associated with Rehospitalisation for Cellulitis and How to Mitigate Against Them

Interviewees: Gavin Barlow[1,2]

1. Senior Clinical Lecturer, Experimental Medicine & Biomedicine, Hull York Medical School, University of York, UK
2. Honorary Consultant, Infection Research Group, Hull University Teaching Hospitals NHS Trust, Hull, UK

Disclosure: Barlow has received an honorarium from Advanz for the reported interview and his involvement in the writing of a related article; and, in the last 2 years, has received an honorarium from Pfizer Vaccines UK for serving on an advisory board.

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Summary

Gavin Barlow, Hull York Medical School, UK, spoke with the EMJ about risk factors for the bacterial infection cellulitis, including prior history of the condition, older age, and a number of comorbidities ranging from diabetes to athlete's foot. Following hospitalisation, readmission for cellulitis, which occurs in around 12% of patients in the first month, may be due to such risk factors, as well as socioeconomic conditions. Readmission can greatly impact healthcare utilisation in economic and infection control terms and the use of sparse facilities, such as side-room beds. It can also impact a patient's quality of life (QoL). Mitigating against readmission for cellulitis necessitates an understanding of the reasons for such, including risk factors, and how best to control them by, for instance, giving proper healthcare provision for comorbidities and educating patients to raise awareness of the recurrence of cellulitis.

INTRODUCTION

Cellulitis is an acute bacterial skin and skin structure infection that is most often caused by β-haemolytic streptococci and *Staphylococcus aureus.* It presents with acute onset of redness, swelling, pain, tenderness, and warmth, most commonly of a lower limb.[1] Risk factors for cellulitis include: a prior history of the condition; older age; intravenous drug use; presence of lymphoedema or an active wound; fungal foot infection; skin conditions; previous limb surgery; obesity; and venous insufficiency.[1-3] Treatment most often involves oral antibiotics for 5 days or longer, as needed in the community, but hospital admission is required when progression occurs despite oral antibiotics.[1]

The EMJ discussed with Barlow the main reasons and risk factors for cellulitis readmissions; the economic, infection control, and QoL factors associated with readmission; and how readmission risk could be mitigated.

REASONS FOR HOSPITAL READMISSION FOLLOWING CELLULITIS

According to Barlow, patients with early readmission following a hospital stay for cellulitis are generally older and may have one or more of the comorbidities associated with cellulitis such as an active wound or leg ulcer, diabetes, heart failure, or uncontrolled skin conditions. Both admission and readmission may also be impacted by socioeconomic factors. "If you've got an older patient in poor social circumstances who's got a lot of comorbidities," Barlow suggested, "then you want to look carefully at them before discharging to ensure you've done everything to mitigate readmission and recurrence."

Barlow stressed that it is key to understand why someone has been readmitted; one point of assessment is to determine whether the readmission is due to a new episode of infection or the persistence of an initial infection as a result of misdiagnosis, sequelae, or the destabilisation of comorbidities. Readmission could also be due to undertreatment. The DANCE trial showed that 24% out of 73 patients hospitalised for cellulitis who were receiving 6 days of antibiotic treatment relapsed within 90 days; however, this figure was only 6% for 76 patients who had received 12 days of treatment.[4] A further reason could be due to non-adherence to oral antibiotic therapy.[5]

Readmission for a new infection tends to occur after a period of at least a few months of improvement or a resolution of symptoms and signs following the initial episode. This, Barlow stressed, was an important distinction, as clinical and preventative management for a new infection compared to a continuing infection or sequelae may differ, and noted that "there's good evidence that the strongest risk factor for recurrent cellulitis is previous cellulitis."

In regard to other bacterial skin and skin structure infections, Barlow reported that "it's very likely that some of the cellulitis risk factors may also be important, particularly older age, socioeconomic status, and comorbidities."

ECONOMIC FACTORS ASSOCIATED WITH HOSPITAL READMISSIONS

A key economic problem identified by Barlow following readmission for cellulitis was hospitalisation costs: "Patients over 70 years of age form about two-thirds of those admitted with major skin infections locally and they often spend several days in hospital." Indeed, analysis of data from the National Health Service (NHS) England Hospital Episode Statistics[6] showed that around 90,000 people per year are admitted into hospital for cellulitis. Of these, approximately 12% are readmitted in the first month, with 21% admitted within 12 months. These readmissions last for an average of 2.8 bed days and are at a mean cost of £1,560 per patient.[6]

While hospitalisation time may be dropping with the use of outpatient parenteral antibiotic therapy in the UK, Barlow added that "sometimes it's difficult for older patients to access outpatient parenteral antibiotic therapy, so they can spend considerable time in hospital." This has particularly been an issue during the COVID-19 pandemic wherever bed occupancy has been high, and there is an ongoing problem due to long waiting lists for elective surgeries. "If a readmission due to cellulitis becomes a medical outlier on a surgical ward," explained Barlow, "then that will potentially have a negative knock-on effect to a patient due to come in for elective surgery."

Also discussed with Barlow were indirect costs: "If the patient is working, there's potentially the cost to that individual and their employer. There are also costs to the family if they have to come and visit or take care of children or a pet."

MITIGATING THE RISK FOR READMISSION

There is a need for knowledge about who is at risk for both readmission and recurrence of cellulitis, and how one might mitigate that. "Early readmission mitigation is more around ensuring the diagnosis is correct, adequate initial therapy, and stabilising social situations and comorbidities," Barlow explained, "recurrence is more about addressing individual risk factors." As having an episode of cellulitis is one of the primary risk factors for a further episode,[2]

patients should be warned to present to a healthcare professional as soon as they feel that they may be experiencing cellulitis recurrence.

"Clearly for early readmission," Barlow highlighted, "it's really important to think of social circumstances that can sometimes contribute and the need for physiotherapy and occupational therapy. That aspect of patient care is important to remember, as well as asking about comorbidities, are they stable, and if not, can their condition be optimised?" This may especially be the case for patients with lymphoedema; there is also evidence that recurrence is less common following compressive therapy.[7]

With these factors in mind, Barlow emphasised the need for ongoing care for cellulitis combined with managing comorbidities. In both primary and secondary care, he explained that "patient and healthcare professional awareness is really important as some of these things are modifiable risk factors for readmission and recurrence. Athlete's foot is relatively easy to treat, but is sometimes overlooked as a risk factor."

A further issue that Barlow underscored was that, generally, most infections are not managed by infection experts. Inexperience in identifying cellulitis may, in some cases, lead to misdiagnosis; for example, confusing deep vein thrombosis or heart failure for cellulitis.[1] Where cellulitis is confirmed, resolving symptoms and signs may also be confused with persisting infection or recrudescence, which can lead to unnecessary readmission and antibiotic therapy.

Also important is the need for general foot and limb hygiene and for professional chiropody and podiatry for patients with nail and foot problems, respectively. Patients with dry skin need to be advised to moisturise and if a patient is known to be colonised with methicillin-resistant *S. aureus* and cellulitis is recurrent. Barlow advised how he would usually try to decolonise them. "Although," said Barlow, "evidence for the success of this is open to debate."

Another potential way to mitigate against rehospitalisation for cellulitis is long-term, low-dose antibiotic use, as it was shown in a meta-analysis by Dalal et al.[8] that 6–18 months of antibiotic prophylaxis can reduce cellulitis recurrence.[8] However, Barlow stressed that "in the context of antibiotic resistance, I don't give long-term antibiotics to all patients with cellulitis, but if you've got someone who's had two or more recurrent episodes within a relatively short period of time, then it's worth discussing with them the pros and cons of antibiotic therapy for 12 months using a shared decision making approach." Barlow also described other interventions, such as using a longer initial course of antibiotics for selected patients at risk of readmission, as suggested by the results of the DANCE trial,[4] and the role of stand-by antibiotics. Another alternative is the use of long-acting injectable antibiotics.[9]

For patients coming into hospital for surgery, Barlow discussed how "pre-operative assessment and stabilisation of comorbidities are important for more complex patients. If they have a particular comorbidity, or they're known to be *S. aureus* skin-colonised, then it's important to decolonise them, depending on the surgery they're undergoing." Care and attention is also required at the time of surgery and with post-operative wound care.

QUALITY OF LIFE FACTORS ASSOCIATED WITH HOSPITAL STAYS

"In my experience, most patients prefer to be at home," relayed Barlow, "as there's a negative effect on QoL for patients in hospital. It's probably much more important than we recognise. For instance, many patients will be in an open bay area, so there may be a knock-on effect on sleep."

Furthermore, this could potentially have a negative effect on mental health, and older patients in particular can become disorientated in hospital. This can lead to a cycle of decline, necessitating more medical interventions. "Patients who stay in hospital for quite a few days can get down," Barlow explained, "and those that stay in hospital for weeks can get very low and depressed." This can especially occur in patients who have two or three admissions for cellulitis in 12 months, or those who suffer sequelae such as lymphoedema.

An additional problem that Barlow highlighted was that "for older patients especially, for every day they spend in hospital they lose a bit of their muscle mass, and strength is really important for health."

INFECTION CONTROL FACTORS ASSOCIATED WITH CELLULITIS

In general, Barlow claimed, patients with cellulitis are not admitted to an infectious disease ward or to a side room in the UK National Health Service (NHS): "In our hospital, 90% of those with cellulitis are managed on general medical wards in open bay areas." As such, Barlow went on to say that "infection control always has to be a consideration and we always make a risk assessment when a patient is admitted with infection." He particularly pointed out the risk of Lancefield Group A *Streptococcus* in a patient with an open or leaking wound, which would be especially worrying for a patient who may not understand the need for contact precautions.

With this in mind, such patients, or those with methicillin-resistant *S. aureus*, may need to be cared for in a side-room. However, this can be problematic as most hospitals have a limited number of side-rooms that may also be needed for other patients, including those with behavioural problems and those receiving end of life care.

CONCLUSION

Around 12% of hospitalised patients with cellulitis will be readmitted within the first month following discharge. This can have a considerable impact on patient QoL, healthcare economics, and infection control. Understanding and addressing the underlying reasons for readmission due to cellulitis may help to reduce such consequences. Further research is needed to understand and mitigate associated risk factors.

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ADV/DAL/PM/0095

Interviews

David Heymann CBE and Ron Daniels shared insights into their careers and spoke of their research in COVID-19 and sepsis.

Featuring: David Heymann CBE and Ron Daniels

David Heymann CBE

Professor of Infectious Disease Epidemiology, London School of Hygiene & Tropical Medicine; Head of the Centre on Global Health Security, Chatham House, London, UK

Q1 Please summarise the primary duties and key projects you undertook while serving as Chairman of Public Health England (PHE).

I began working in the Health Protection Agency (HPA) in 2009 and after a period of time that transferred to PHE. This was in the midst of the swine flu pandemic and, therefore, being able to deal effectively with such outbreaks was one of our key priorities. Upon becoming Chairman of PHE, I was given a number of assignments. One of these was to increase the global footprint of the agency. It is important to understand that health is global, and health security for the UK can best be guaranteed by strengthening the capacity of countries around the word. Consequently, one of my tasks was to strengthen the international activities of the HPA and then PHE. We also strengthened the national public health capacity by setting up the Field Epidemiology Training Programme, a 2-year mentorship programme that trained new people in PHE on public health outbreak investigation and response. Lastly, since PHE had been brought together from over 70 different groups in the UK, it was essential to make the agency more corporate in its budgeting and work activities. This is something else I was directly responsible for.

Q2 Please could you tell us about your roles during the current COVID-19 pandemic as well as past human coronavirus outbreaks?

I have mainly been exposed to the current pandemic as Chair of the World Health Organization (WHO) Strategic and Technical Advisory Group on Infectious Hazards (STAG-IH), and through relationships in the UK established during my time as Chair of PHE. Since stopping chairmanship in 2017, I remain in touch with colleagues at PHE, now the UK Health Security

Agency (UKHSA). I am also a Professor of Infectious Disease Epidemiology at the London School of Hygiene and Tropical Medicine, UK, where we often have discussions with modellers and others. As Chair of the WHO Strategic and Technical Advisory Group for the Emergencies Programme, I have been in regular contact with various aspects of the pandemic and response activities in countries around the world. I also participate in faculty activities at the National University of Singapore.

In 2003, while I was on secondment to WHO from the Centres for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, I was in charge of the communicable diseases area and led the WHO response to the severe acute respiratory syndrome outbreak. When the Middle East respiratory syndrome coronavirus outbreak occurred, I was in the UK at PHE. I travelled to Saudi Arabia several times during this period to work with the PHE team and the Saudi Arabians to gain a better understanding of that infection.

"It is clear from this pandemic that there are three interlocking health functions that must be addressed in preparedness going forward."

Q3 Could you highlight the principal findings and wider relevance of the recently published article you co-authored, entitled 'Disease Surveillance for the COVID-19 Era: Time for Bold Changes'?

Governments need to understand that surveillance is a vital part of their healthcare activities. This is especially important in the context of COVID-19 because, in addition to looking for infections, governments must also look for variants, which involves genetic sequencing. An increasing number of countries are gradually beginning to develop this capacity. For example, handheld genetic sequencers are being provided to developing and lower-middle-income countries by the Wellcome Trust and Bill and Melinda Gates Foundation, along with training on their use. This will revolutionise the way surveillance is conducted in the future. It is important that surveillance information is collected, used nationally and then shared globally. This will require government commitment, high technologies using the best possible tools available, and having the technical support necessary to do that.

Q4 What can healthcare systems learn from the COVID-19 pandemic in order to become more resilient the next time a major public health emergency appears?

It is clear from this pandemic that there are three interlocking health functions that must be addressed in preparedness going forward. The first is stronger public health, including detection systems, response systems, and systems of prevention and working in a 'One Health' environment. The second is resilient health systems that can take care of the surge of patients who might be infected in a pandemic whilst also continuing routine activities. The third function relates to the fact that people with comorbidities have been more severely affected by COVID-19. Consequently, it is imperative that healthy lifestyles are enabled. This will ensure populations are better able to resist infections in the future.

Q5 How important do you believe the One Health approach will be in preventing and preparing for future pandemics?

After the severe acute respiratory syndrome outbreak in 2003, the Chinese conducted several studies in their animal farms and found an antibody suggestive of coronavirus infection at some sites. They also looked in live-animal

market workers and determined that, overall, 13% had an antibody to coronavirus, as opposed to approximately 1–3% of individuals in the general population. At that point in time, had the policymakers in China taken the messages to heart, they might have been able to implement education programmes in the markets, develop vaccines for animals that they raise on their wild animal farms, and introduce some other measures that may have helped in preventing subsequent pandemics. I'm not saying it would have, but it is always important to apply the knowledge that you have in a way that can prevent future events.

Q6

You have published over 200 peer reviewed articles, commentaries, and book chapters. What do you believe to be the current knowledge gaps with respect to monkeypox?

Monkeypox was first identified in laboratory animals in Denmark in the 1950s. The first human cases were identified in sub-Saharan Africa in the 1970s, at a time when smallpox was on the wane. After the eradication of smallpox in 1980, there was concern that human monkeypox might replace smallpox. This was because the smallpox vaccination protected against monkeypox. Therefore, several surveys were performed in children under 15 years of age without smallpox vaccination scars, looking for facial scars or antibody in the blood that might be an orthopox. Results showed that there was very little infection, and so it was not believed that monkeypox could replace smallpox at that point. Since then, more and more cases of human monkeypox have been observed. There are two different clades of virus: one which has a higher case fatality rate of approximately 10% in the Democratic Republic of Congo and Central Africa, and one with a lower case fatality rate in West Africa. Countries in West Africa, especially Nigeria, seem to be experiencing an increase in the number of cases. These cases appear to be simple zoonotic infections, and do not transmit from human-to-human very easily. However, studies are now being conducted to determine whether there might be transmission asymptomatically. In the Democratic Republic of Congo, there have been several major outbreaks. Notably, most transmission is occurring in people who have never been vaccinated. This means that people under the age of 30 or 40 years are having sustained outbreaks of monkeypox. In summary, there is still a lot that remains to be elucidated about monkeypox, and it is still not clear whether this virus could one day fill the epidemiological niche left by smallpox.

Q7

To date, what have been the proudest achievements of your distinguished career?

I can't claim any of those achievements myself because there were always teams involved. Early on, I was fortunate to work in a very successful public health programme, namely the smallpox eradication programme. The people with whom I worked at the time have remained my support network and colleagues. Although I was a small cog in the wheel, my very first activity in public health was very satisfying, particularly because of the long-term outcome of having eradicated a disease.

Q8

Where can we expect to see your focus lie in the near future?

My focus will be to continue providing what knowledge and skills I can to others, both as a mentor and through working on various advisory groups in the WHO. I will be teaching more than I have in the past at the Saw Swee Hock School of Public Health, National University of Singapore, in Singapore and the London School of Hygiene and Tropical Medicine, UK. Finally, I shall continue giving classes at the University of Oxford, UK; Harvard School of Public Health, Boston, Massachusetts, USA; Erasmus University Rotterdam, the Netherlands; and at various other European and North American Universities. ■

Ron Daniels

Chief Executive and Founder, UK Sepsis Trust;
Consultant in Critical Care, University Hospitals Birmingham NHS Foundation Trust, UK

Q1 What does your role as Chief Executive of the UK Sepsis Trust entail, and what have been your greatest achievements to date in this position?

I first developed an interest in sepsis in 2004, when I watched a young male, a 37-year-old, die of sepsis in my intensive care unit. It became apparent that the system had let them down, at multiple opportunities, to recognise this illness. We had not taken proper observations and had failed to provide adequate safety netting to his family. Their death was almost certainly avoidable. For several years after, I started to develop clinical tools for health professionals and engage with hospitals. This ultimately led to a groundswell of support among medical practitioners. In addition, we developed the Sepsis Six care bundle and managed to get this implemented in approximately 100 hospitals across England by 2010. However, it became apparent that this was only part of what was needed in terms of health professional education. It was also necessary to work with the public and empower them to access healthcare rapidly. Furthermore, we needed to work with policymakers to ensure that, at a national level, sepsis was recognised as a clinical priority.

Over the 2-year period leading up to 2012, we founded and eventually registered the UK Sepsis Trust. Since that time, as the Founder and Chief Executive, I have led the charity. I now co-lead it with Sarah Hamilton-Fairley and dedicate 27 hours of my time each week to that leadership role. Within the charity, I am responsible for setting the strategic direction. I oversee both the clinical and campaign sides.

In the clinical space, I lead the support team, which is a team of senior intensive care nurses who have received training and counselling. These nurses provide telephonic and email support and, outside of the COVID-19 pandemic, they offer face-to-face peer support groups to people affected by sepsis.

The second area of clinical work involves continuing the process that I started in 2004. This relates to the iteration of the clinical tools for health professionals and the provision of education, lectures, and design of e-learning. Politics is the third area of clinical work I am concerned with. This encompasses the development of relationships and collaborative working, often with multi-stakeholder groups, to refine national guidance and ensure that it is fit for purpose. One example would be the support we gave to the National Institute for Health and Care Excellence (NICE) when they released NICE guideline NG51. In the campaign space, we prioritise empowerment. Essentially, we empower the public to ask: "Could it be sepsis?" I co-design the tools with Hamilton-Fairley, we promote them to people who can activate them, and we form networks to disseminate at scale. We worked closely with the frozen food retailer Iceland, who put messages raising awareness of sepsis and its symptoms on 100 million milk bottles. There was no cost to the supermarket because milk labels need to be printed anyway. They simply chose to put potentially life-saving information on them.

In terms of major achievements, it's difficult to pick with precision the one we are most proud of, especially because the charity is now entering its 10th birthday year. However, I think we can safely say that we have put sepsis on the map for the National Health Service (NHS), meaning that it has become a clinical priority. We lobbied, through the Department of Health and Social Care (DHSC), to ensure that sepsis was a commissioning incentive, and NHS England actually applied a Commissioning for Quality and Innovation incentive. Hospitals were asked to screen patients for sepsis and, once

they suspected sepsis, treat them rapidly with antibiotics. This resulted in significant process improvements, saving hundreds of lives without the adverse consequence of increasing total antibiotic usage and fuelling the development of antimicrobial resistance. A second success was the NICE clinical guideline NG51, produced in 2016. This was a ministerial mandate that paved the way for a unified pan-UK approach to sepsis. In the near future, the Academy of Medical Royal Colleges (AoMRC) is going to update that advice and release new guidelines.

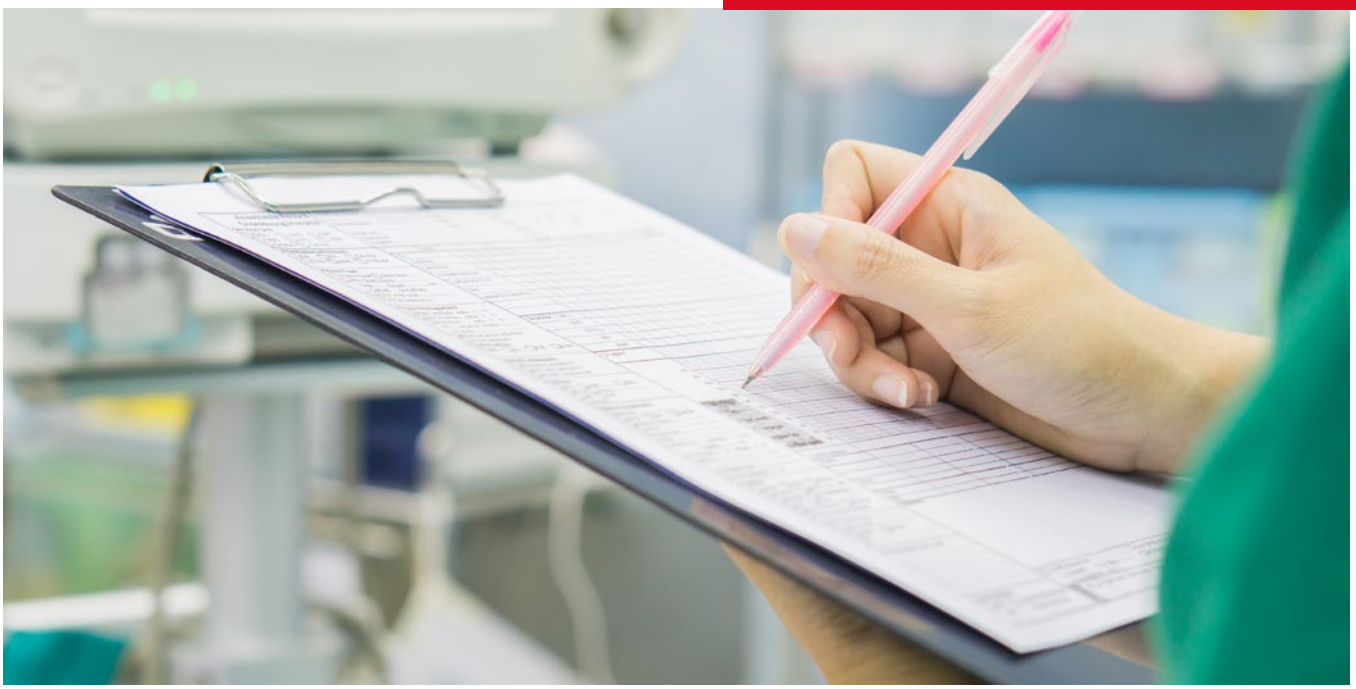
In summary, we can be very proud that we have increased awareness among health professionals. At a policy and statutory body level, we secured lasting changes that will help ensure healthcare organisations can continue prioritising sepsis. We also heightened public awareness of the condition. In our early polls, approximately one in three members of the public had heard of sepsis. Prior to the pandemic, we were in a position where 80% had heard of sepsis. Of that 80%, over two-thirds knew what to do about sepsis, recognised it as a medical emergency, and were aware of how to access medical help. I think this combination of factors has ensured that we are saving lives. Importantly, we are also improving outcomes for survivors. Our nurses are available during office hours, and they support hundreds of sepsis survivors every year, helping them understand the recovery journey, become engaged in the recovery process, and improve their quality of life.

Q2 The goal of the UK Sepsis Trust is: "To end preventable deaths from sepsis and improve outcomes for sepsis survivors." In your opinion, what research priorities should be set to achieve this?

We have to acknowledge and reinforce that not every death from sepsis is preventable. Sepsis can be a mode of death in people approaching the natural end of life. Of course, where mortality from sepsis is avoidable, it is important that we understand how to prevent deaths in that situation in the future.

The main research priority is a top-level one, which will not be delivered by a single piece of research. It is around moving towards precision medicine. Currently, there is one definition of sepsis, which applies the same thresholds regardless of whether a patient is a healthy 18-year-old or a frail 85-year-old. This is illogical because the phenotype, vital signs, and laboratory parameters of the 18-year-old, who requires urgent antibiotics and source control, are likely to be very different to those of the 85-year-old, who needs similar.

The main research priority is a top-level one, which will not be delivered by a single piece of research. It is around moving towards precision medicine.



Depending on the set of comorbidities, age, and type of infection, certain individuals will need to be given immediate antimicrobials. However, in other individuals, it might be feasible to wait until more information is accrued before administering therapy. To differentiate between these groups, patient-level data on a large scale is required. Such data systems must be intraoperable and pattern recognition strategies should be applied to them. Only then will it be possible to understand what sepsis looks like at an individual patient level. This will ultimately allow clinicians to use antibiotics more judiciously and prioritise those who need them most urgently. It also paves the way for further research into immunomodulatory therapies, which can help health professionals deliver precision medicine to the patient.

Q3

Please could you provide an overview of the Sepsis Six treatment pathway? What are the potential facilitators and barriers towards implementing this care bundle?

The Sepsis Six is a set of interventions developed and curated by the UK Sepsis Trust. It takes evidence-based guidelines that are, by necessity, very thorough and it operationalises them into a simplified bundle. The pathway consists of the six steps of care that, at present, academic experts and key opinion leaders suggest are the most important. We seek to work with stakeholders such as NICE to ensure that there is a degree of endorsement for the bundle and that it is delivered responsibly and rapidly. Step one is to ensure that a senior clinician attends the patient. This is important because senior clinicians can expedite and facilitate care. They can also look for non-sepsis conditions that mimic sepsis, and move patients onto alternative pathways if necessary. Step two is to correct hypoxia (e.g., administering oxygen if a patient's oxygen saturation is low). Step three is to secure intravenous access and send a full set of blood tests. In this instance, we are looking for organ dysfunction, we are looking to risk stratify, and we are looking for pathogens. This enables us to tailor antimicrobial therapy. Step four is to control the infection. This is achieved by giving antibiotics and, if appropriate, source control (e.g., removal of an infected intravascular device or surgery for an abscess that might be precipitating sepsis). Step five is to consider fluids. Intravenous fluids may be given, particularly if the patient shows evidence of shock. Finally, step six is ongoing monitoring (e.g., continue monitoring the National Early Warning Score [NEWS] 2, urine output, and lactate).

Collectively, this package of care is now in use in at least two dozen countries around the world. In part, this is because it is empowering; simple; and directed towards junior health professionals, providing them with a tool they can readily utilise at the bedside. As mentioned above, I think the biggest enabler of Sepsis Six is its empowerment value. It is memorable, logical, and allows junior staff to act rapidly when faced with deteriorating patients. There are two main barriers. In low- and middle-income countries as well as resource-constrained high-income countries, there are issues with access to the equipment that is needed to deliver the interventions. This could range from the availability of blood culture bottles through to how antibiotics are stored, how they are accessed by health professionals, and whether there is a prescriber available. Issues with availability also include access to point-of-care testing, for example, to measure lactate.

In my opinion, the biggest barrier remains the perception that delivering reliable and rapid sepsis care negatively impacts antimicrobial resistance. Over the last decade, there has been a profound change within the NHS from a culture of wanting to rapidly treat sepsis to an appreciation that if we overtreat it, then we are likely to exacerbate the spread of antimicrobial resistance.

We are now encouraging organisations to look at their sepsis protocols, make sure that they are compliant with the NICE clinical guideline NG51, and, in the near future, to make sure they are compliant with the AoMRC guidance. We also advise that organisations empower their junior staff to act but do not mandate their actions. If a member of staff exercises clinical judgment and has doubt that sepsis is the diagnosis, then they should equally be empowered to withhold antibiotics, seek senior help, or wait for investigations to come back in a timely fashion.

Q4

How important is the clinical concept of Red Flag Sepsis in the identification of patients at high-risk of sepsis-related mortality?

Red Flag Sepsis is a tool of empowerment; however, it is not intended to replace clinical

judgment. Red flags are there for patients about whom we are not sure what to do. They are parameters that can quickly and easily be identified at the bedside, and are intended to empower the often junior health professional to act decisively and rapidly. Contextually, there is an international consensus definition of sepsis, but it is a definition that requires a full set of laboratory tests available at the point of decision, together with an understanding of intensive care terminology. Operationally, the reality is that the Sequential Organ Failure Assessment (SOFA) score, which is this official definition of sepsis, is impracticable at the bedside. This is where red flags come into their own.

Q5

Could you summarise the principal findings and wide relevance of the 2019 article that you co-authored, entitled 'Life After Sepsis: An International Survey of Survivors to Understand the Post-sepsis Syndrome'?

The first thing to say about this study is that it was a survey, and it therefore comes with all the associated caveats. We almost certainly had selection bias in the patients who reported in to us. It is highly unlikely that somebody who survived sepsis, has remained well, and returned to normal life is going to respond to such a study. However, with those caveats in place, the findings were unsurprising, and they mirrored other evidence. Forty percent of people who survived sepsis have a deficiency in one of three domains persisting at 1 year after their original illness. The three domains are psychological, physical, and cognitive. The psychological domain ranges from relatively mild (e.g., difficulty sleeping, the occasional panic attack, or heightened anxiety) through to severe (e.g., post-traumatic stress disorder, which occurs in approximately one in five survivors of sepsis). The physical domain ranges from the very visible (e.g., the loss of digits or limbs) through to the equally disabling invisible physical sequelae (e.g., the severe pain that people can experience in their limbs and joints, and the more common but equally disabling fatigue). Similarly, the cognitive domain ranges from the relatively minor (e.g., poor short-term memory or concentration) to the disabling (e.g., where an individual's judgment and ability to conduct basic tasks is impaired). Collectively, these symptoms cause a reduction in the patient's attitude, can be detrimental to their mental health, and can compromise their ability to return to work at their previous level of function. Their ability to manage and sustain relationships, including in the home, can also be impaired. Overall, these findings were not surprising. However, certain elements health professionals might traditionally have perceived as trivial were shown to cause significant problems to patients. These elements included brittle hair and nails as well as the sensation of loose teeth. Also of interest are the results of a previous Scandinavian study. At 1 year after infection with sepsis, 57%

of previously employed adults had returned to work. However, 43% had not. I would like anyone accessing this resource to consider how the inability to return to work because of the after-effects of sepsis would impact on their own lives.

Q6 Have there been any recent innovations in the development of rapid diagnostic tests for sepsis that you believe are particularly noteworthy?

The challenge with sepsis is that it is so heterogeneous. We could have a young person with a urinary tract infection who presents to healthcare within 12 hours of their first symptoms. Alternatively, we could have an older person, with more comorbidities and significant risk factors for infection, who develops pneumonia and delays accessing healthcare for 48 hours. For this reason, it is unlikely that a single biomarker or pathogen identification test will provide a transformational answer.

I have previously referred to the need for big data and precision medicine. However, I think the biggest transformation we are going to see over the coming years is a move towards diagnostics being closer to the patients. This will include risk stratification diagnostics, which might involve lactate being more available, alongside other risk stratification tools, such as point-of-care assessment of kidney and liver function. It will also include pathogen identification strategies and molecular techniques for the rapid identification of pathogens, which are coming increasingly to the fore. It is anticipated that these will no longer be subsumed within a laboratory, which delays their impact on the decisions of the prescribing clinician. Increasingly, these are going to be integrated more closely into the healthcare system and accessible at the point of care.

"The main research priority is a top-level one, which will not be delivered by a single piece of research. It is around moving towards precision medicine..."

Q7 Why is it important to standardise national track and trigger systems across primary and secondary care?

When we talk about track and trigger systems within the UK for adults, particularly non-pregnant adults, we are primarily talking about the NEWS, which is now in its second incarnation. This has a significant evidence base in terms of the detection of deterioration in acute care in the secondary sector, and has an increasing evidence base in terms of its utility in the pre-hospital and community-based phases, including in general practice. I think it is important that we have a standardised tool. When referring a patient about whom they are concerned, a standardised language could support a general practitioner in dialogue with the receiving medical officer at the hospital. They can convey, quickly and easily, the level of deviation of that patient's deterioration from normal in a language that the receiving medical officer can retain and understand. In obstetrics, we ought to move towards a standardised Obstetric Early Warning Score (OEWS). There is already work under way in standardising the Paediatric Early Warning Score (PEWS). Although it is important to have a common language, there are two aspects that we need to caveat this with. One is that the tools do not replace clinical judgment. The tools are not yet perfect and if clinical judgment suggests that the patient is unwell, despite the patient not triggering on a particular tool, then clinical judgment should be trusted and action should still be taken. I think the second caveat is that, just like the definitions for sepsis, it is important to understand that applying physiological thresholds at the same level to people of widely different ages, widely different baseline functions, and with widely different collections of comorbidities is likely to be imprecise. As we understand in a more granular and cohort-specific way what deterioration looks like for a particular group of patients, we can anticipate that early warning scores might become customised according to the baseline risk factors of the patient.

Q8 You were on the Scientific Committee of the World Sepsis Congress (WSC) 2021. Please could you provide a brief summary of the key take-home messages from this event?

One of the biggest attractions of online congresses, providing that people can access mobile data, is that they are accessible to individuals in low- and middle-income countries

in a similar way to being accessible to people in high-income countries. One of the greatest learnings we had from this was that the engagement of people in resource-poor nations was both relatively straightforward and also hugely appreciated by the clinicians working in those settings, who would normally find it difficult to access key opinion leaders, particularly from high-income countries around the world.

Another learning from the WSC was that sepsis is a whole systems issue. It is not around an individual biomarker, it is not just around pathogen identification, and it is not around secondary care only or community-based care only. It is about the healthcare system as a whole. This includes the public health aspects of the healthcare system (e.g., making the public aware of sepsis and emphasising the need to access healthcare urgently) and also integration between community-based care and secondary care in order to speed up access to hospitals, facilitate rapid recognition of sepsis, and promote the timely delivery of life-saving antimicrobials.

On the subject of antimicrobials, it is key to understand that sepsis is intrinsically interlinked with antimicrobial resistance. There is an increasing drive towards a more holistic approach by governments and policy makers with respect to infections management, which is based on four pillars: antimicrobial resistance, rapid treatment of sepsis, infection prevention, and pandemic preparedness. It is imperative that these four pillars are all addressed with equal vigour.

Another learning from the recent congress was around the direct relationship between severe acute respiratory syndrome coronavirus 2 infection, COVID-19, and sepsis. The majority of individuals who became critically ill with COVID-19, particularly in high-income countries, had sepsis precipitated by a viral cause, and this is really important to understand.

Finally, there was a consideration of exciting future developments. I have already mentioned several of these in my answers above. They include the development of more intelligent and customised biomarkers to guide care; the development of pathogen identification strategies; and the development of regional research networks, such as the proposed Pan-European Sepsis Network, to drive the research agenda, elucidate the burden of sepsis on society, and understand how we can move toward precision-based medicine.

Q8 Please could you outline the primary duties and key projects you undertake as Vice President of the Global Sepsis Alliance (GSA)?

The GSA is based on multistakeholder engagement. We have regional offices that broadly geographically mirror the World Health Organization (WHO) regional offices. It is about facilitating collaborative work in often challenging regions, such as the African Sepsis Alliance (ASA). It is also around public engagement. For instance, World Sepsis Day messaging is used to nurture events related to sepsis in countries across the globe. At least 80 countries now have World Sepsis Day events every year, which engage clinicians, members of the public, and policymakers.

Of course, there is the strategic policy engagement. We cannot talk about the GSA without mentioning the WHO. In 2017, we presented a resolution to the WHO, proposing that countries heighten their public awareness of sepsis; ensure robust infection prevention strategies are implemented through vaccination, clean water, sanitation, and hygiene; and that they strengthen the resilience of their healthcare systems against sepsis. It is also important for countries to measure their performance against sepsis metrics. Although the resolution was adopted, progress has been hindered by the pandemic. However, as we move into 2022, we are looking forward to an implementation task force, developed jointly with the WHO, in order to consider the recommendations within the resolution and start to work across the regions to activate them.

The final thing is around the WHO being made aware of the infection management strategy, considering the holistic approach to infections management, and being made aware of the Infection Management Coalition (IMC) White Paper. This report was produced as a collaborative stakeholder strategy in the UK and has now gone to the top of the WHO. Hopefully, it will establish a template for governments to plan and deliver a more cohesive approach to infection management at a policy level. ■

SEPSIS IN NUMBERS

STATISTICS

48.9 million cases in **2017**

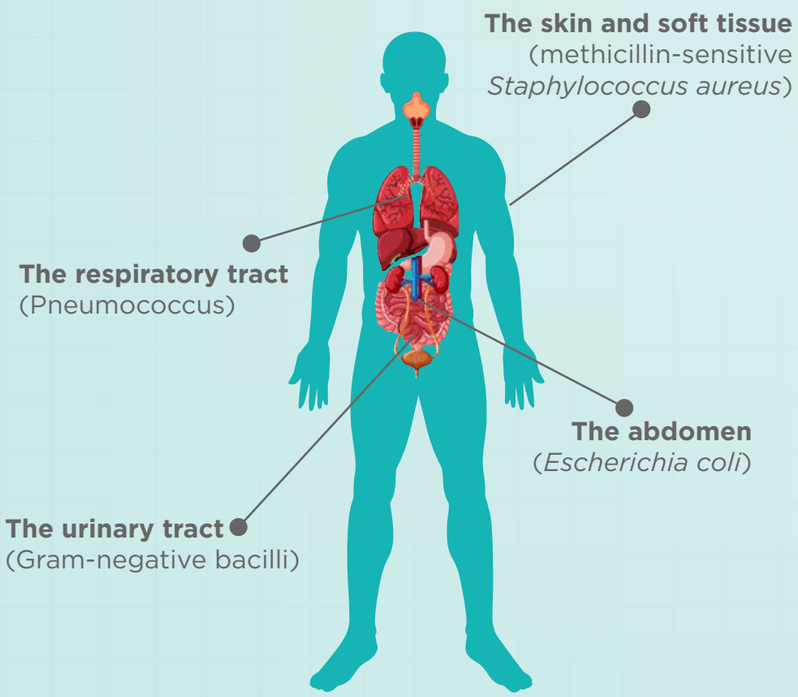
11 million sepsis-related deaths globally in 2017

3.4 million individuals develop sepsis each year in **Europe**

Nearly 700,000 deaths per year in Europe

CAUSES

Common sites of infection and causative microorganisms



WHO IS AT RISK?

More likely to occur in:

- Neonates
- Older adults
- People with chronic medical conditions
- Immunosuppressed patients
- Pregnant and recently pregnant females

SCREENING

Tools facilitating early identification in patients with known or suspected infection

Sequential Organ Failure Assessment (SOFA)

Based on oxygen levels, Glasgow Coma Scale score, mean arterial pressure, bilirubin level, serum creatinine level, and platelet count

Quick Sequential Organ Failure Assessment (qSOFA)

Score of ≥2. Based on low systolic blood pressure (≤100 mmHg), tachypnoea (≥22 breaths/min), and altered mentation (Glasgow Coma Scale <15)

Systemic Inflammatory Response Syndrome (SIRS) Criteria

Based on fever (>38 °C) or hypothermia (<36 °C); heart rate >90 beats/min; respiratory rate >20 breaths/min; and leukopenia (>4,000 white blood cells/mm^3) or leukocytosis (>12,000 white blood cells/mm^3)

Modified Early Warning Score (MEWS)

Based on consciousness level, hourly urine output, respiratory rate, systolic blood pressure, and temperature

MANAGEMENT

- Administer antimicrobials, ideally within 1 hour of sepsis recognition
- Source control, within 6–12 hours of diagnosis
- Crystalloids as first-line fluid for resuscitation
- For patients who require vasopressors, initially target a mean arterial pressure of 65 mmHg. Norepinephrine recommend as the first-line agent

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Are Positive Urine Cultures Directly Correlated with Elevated Levels of Novel Biomarkers for Childhood Urinary Tract Infection?

EDITOR'S PICK

My choice for the Editor's Pick this issue is this highly relevant article by Uwaezuoke et al. The identification of more accurate biomarkers for paediatric urinary tract infection could facilitate diagnosis and minimise inappropriate antibiotic treatment in children with this condition. In this narrative review, the authors summarise novel biomarkers and the extent that they capture positive urine cultures. This paper adds to the growing body of literature on biomarkers in urinary tract infections and will be a stimulus for the EMJ readership.

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Abstract

Urinary tract infection (UTI) in children is one of the most common bacterial infections that propels inappropriate antibiotic use. Long-term, potentially fatal complications can occur if not properly treated. Prompt investigation and appropriate treatment would prevent these complications. Although urine culture remains the gold standard investigation for UTI, its process is cumbersome and requires time (24–72 hours). Hence, there has been growing interest in the use of urinary biomarkers. However, some conventional urinary biomarkers detected on urinalysis have poor sensitivity values when used singly as a screening tool. Thus, the searchlight has shifted to the role of novel biomarkers in UTI diagnosis. This narrative review aimed to determine if elevated levels of these biomarkers directly correlate with positive urine cultures. A positive correlation may imply that these biomarkers

could serve as novel UTI diagnostics and thus augment urine culture requests. Established and recent serum and urinary biomarkers show disparate predictive abilities for UTI and its related complications. Some have elevated differential levels in upper and lower UTI or febrile and non-febrile UTI. All studies that investigated these biomarkers established culture-positive UTI, highlighting a direct correlation between positive urine cultures and increased concentrations of the biomarkers in body fluids. Because certain uropathogens were less likely to be associated with pyuria, the sensitivities of some neutrophil-related novel biomarkers (such as urine neutrophil gelatinase-associated lipocalin and human neutrophil peptides 1–3) were reduced in cases of UTI caused by these bacteria. While levels of these novel biomarkers directly correlate with positive urine cultures, it appears that there is yet no standalone biomarker with the optimal sensitivity and specificity for UTI. Although these novel biomarkers are promising, translating their measurements into clinical practice with specific clinical utilities will take time. Novel methods interrogating high-throughput serum (and urine) metabolome data with positive urine cultures in a platform-agnostic manner (metabolome-wide approach) will help confirm and identify novel biomarkers that might capture specific aetiologic agents or shared pathways of related agents. The authors recommend that future research on UTI diagnostics should specifically focus on identifying highly sensitive and specific standalone novel biomarkers that can be easily applied as a point-of-care investigation.

INTRODUCTION

Urinary tract infection (UTI) refers to any infection of the upper urinary tract (pyelonephritis) or the lower urinary tract (cystourethritis) by uropathogens and is one of the most common bacterial infections that propels inappropriate antibiotic use in children. Thus, UTI contributes significantly to childhood morbidity and mortality.[1] For instance, acute pyelonephritis may result in renal parenchymal scars in approximately 10–30% of children with febrile UTI if not well treated.[2] The clinical trajectory of these scars comprises hypertension, chronic kidney disease, and eventually, end-stage kidney disease.[3] To circumvent these complications, any suspected case of UTI in a child should be promptly investigated and adequately treated.

The gold standard for investigating childhood UTIs remains urine culture. The definition of positive urine culture is predicated upon specified colony counts of a uropathogen cultured from urine specimens obtained by different methods from a paediatric patient. The paradigm for UTI diagnosis is generally accepted as the growth of a single uropathogen at a concentration of $>10^3$ colony-forming units (CFU)/mL, $>10^4$ CFU/mL, or $\geq 10^5$ CFU/mL from urine specimens obtained by suprapubic aspiration (SPA), catheterisation, and midstream clean-catch, respectively.[4] However, the American Academy of Pediatrics (AAP) revised guideline for childhood UTI proposes a combination of bacteriuria with or without pyuria and bacterial growth of $\geq 5x10^4$ CFU/mL of a single uropathogen from either SPA or catheter urine specimens as the diagnostic parameters.[5] Interestingly, the AAP recommendation differs from the guideline put forward by the National Institute for Health and Care Excellence (NICE). The latter recommends the combination of urinary biomarkers (such as nitrite and leukocyte esterase) and colony count of any Gram-negative bacillus or $>10^3$ CFU/mL of a Gram-positive coccus (from SPA urine specimen), or $>10^4$ CFU/mL of a single uropathogen (from a catheter urine specimen), or $\geq 10^5$ CFU/mL of a single uropathogen (from a midstream urine specimen).[6]

Given the different positions adopted by the two guidelines on the diagnostic utility of some conventional urinary biomarkers (like pyuria, nitrite, and leukocyte esterase), their suboptimal predictive value or sensitivity when used alone has necessitated the searchlight on novel biomarkers as alternative diagnostic tools.[7] These biomarkers have been found helpful in the diagnosis of UTI,[8-10] in predicting its complications,[11-14] and in discriminating between febrile UTI (acute pyelonephritis) and lower UTI.[15]

Based on published evidence, a positive correlation of a biomarker with positive urine culture may imply its diagnostic utility and augmentation with urine culture. Therefore, this narrative review summarises the novel biomarkers and the extent to which they capture

positive urine cultures. Relevant descriptors ("urinary tract infection," "children," "novel biomarkers," "urine culture," and "correlation") were used. The authors searched two electronic databases (PubMed and Google Scholar) for relevant articles published in English or translated into English. Additional information was gleaned from any review articles published on the subject.

ESTABLISHED NOVEL BIOMARKERS IN URINARY TRACT INFECTION DIAGNOSTICS

Although several novel biomarkers have now been adjudged potentially valuable for the diagnostic evaluation of bacterial UTI in children, procalcitonin and ILs are prominent among them.[7] For instance, the uropathogenic *Escherichia coli* (including other Gram-negative bacilli and Gram-positive cocci) interacts with the uroepithelial cells and the peripheral blood monocytes and triggers the pathophysiologic pathways that result in elevated levels of these biomarkers in urine and serum, respectively (Figure 1). First, the infecting strain of *E. coli* is equipped with P fimbriae, which promotes its adherence to the mucosal lining of the urinary tract. After the uropathogen has attached to the uroepithelial cells, an inflammatory response occurs, including IL-6 and IL-8 secretion.[16] While IL-6 elicits the febrile and acute phase responses seen in acute pyelonephritis, IL-8 facilitates chemoattraction of neutrophils to the urinary tract mucosal site. The build-up of neutrophils contributes to the onset of pyuria. The mucosal IL-8 response triggered by *E. coli* underscores the significant contribution of the uroepithelial cells to the IL-8 levels during UTI.[17] Second, an *in vitro* study showed that the same uropathogen also stimulates the peripheral blood monocytes to produce IL-1α, IL-1β, IL-6, IL-8, and TNF-α.[18] These proinflammatory cytokines (especially IL-1β, IL-6, and TNF-α) then send signalling information to the parafollicular (C cells) and neuroendocrine cells to produce procalcitonin. Its level rises in response to this bacteria-induced proinflammatory stimulus and represents an acute phase reactant, as well as a biomarker of severe bacterial infection.[19] Fundamentally, a bacterial infection induces a substantial increase in the expression of the gene encoding calcitonin 1, leading to the production of procalcitonin in all of these differentiated cell types.[20]

Several published reports indicate that procalcitonin and ILs are reliable biomarkers in diagnosing UTI in children. In one observational analytical study, the investigators noted higher levels of IL-6 and IL-8 in the serum and urine of children with febrile UTI than in those of their cohorts with asymptomatic bacteriuria.[21] They also observed that urine IL-6 and IL-8 levels directly correlated with urine leukocyte counts (pyuria) and acute phase reactants like C-reactive protein (CRP) and erythrocyte sedimentation rate. The children's age, gender, and bacterial properties (such as the possession of P fimbriae) also influenced the urine levels of these cytokines. Thus, it is apparent that host- and bacteria-related factors affect cytokine responses in UTI and the presence of pyuria. Some studies reported a lower risk of pyuria in childhood UTI aetiologically attributed to *Enterococcus* species, *Klebsiella* species, and *Pseudomonas aeruginosa*, and a higher risk of pyuria in uropathogenic *E. coli*-related cases.[22]

In a related comparative study, both serum and urine IL-6 and IL-8 levels were significantly elevated in children with acute pyelonephritis than in their counterparts with lower UTI as well as in uninfected controls.[23] Serum and urine IL-6 exhibited higher sensitivity and specificity in diagnosing acute pyelonephritis than serum and urine IL-8. This finding was corroborated in another study, where infants with acute pyelonephritis had higher serum IL-6 levels than their cohorts with lower UTI.[24] These findings underscore the crucial role of IL-6 in the upregulation of serum procalcitonin production seen in acute pyelonephritis and their discriminatory capacity for upper and lower UTIs.

More importantly, there is consistent evidence across several studies indicating that serum procalcitonin levels were significantly higher in children with acute pyelonephritis than in those with lower UTI.[25-28] Additionally, some studies demonstrated the prognostic utility of this biomarker for UTI. High serum procalcitonin levels were strongly predictive of high-grade vesicoureteral reflux in children with the first episode of febrile UTI.[11,29,30] The confirmation

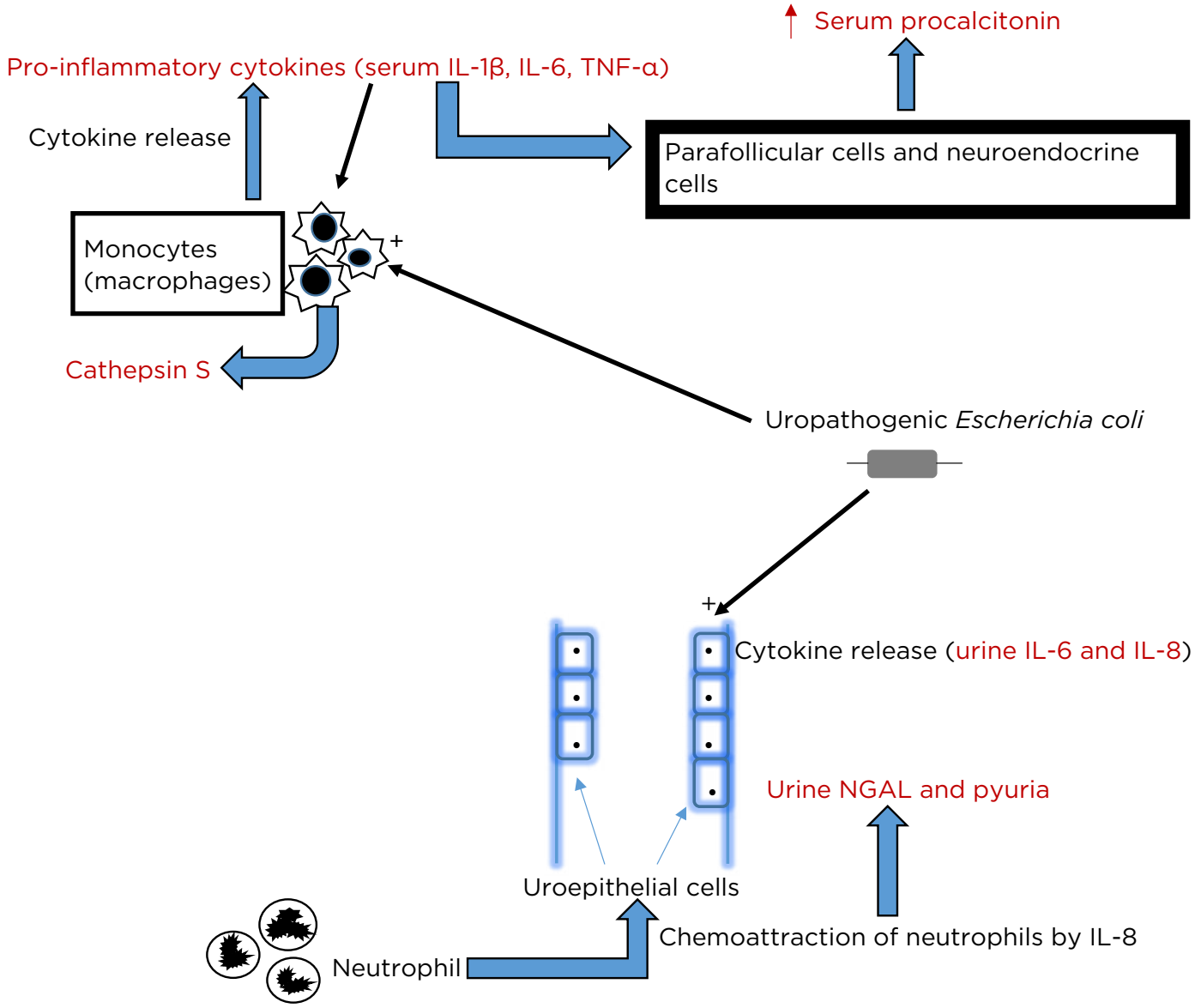

Figure 1: Schematic illustration of the pathophysiologic basis for the elevation of biomarkers in urinary tract infection.

NGAL: neutrophil gelatinase-associated lipocalin.

of this finding precludes the use of imaging studies in low-risk patients. Elevated serum procalcitonin strongly predicted the presence of renal scars in children with culture-proven UTI seen at admission and was even more specific than CRP and pyuria in identifying those children at risk for this complication.[31] Similarly, a hospital-based prospective study showed that serum procalcitonin was an early predictor of renal parenchymal damage in children with the first episode of febrile UTI.[32] This observation makes the biomarker a reliable tool for prognostication in UTI.

Finally, urine neutrophil gelatinase-associated lipocalin (NGAL), urine IL-1β, and urine 8-hydroxy-2'-deoxyguanosine (8-oxodG) are other novel biomarkers that are useful in the diagnosis and prognosis of childhood UTI.[14,33,34] Whereas urine NGAL exhibited high sensitivity for diagnosing UTI,[33] urine 8-oxodG predicted renal parenchymal damage in children with UTI.[14] Furthermore, urine IL-1β was a valuable biomarker for the early diagnosis of febrile UTI in infancy and late childhood.[34] In summary, the novel biomarkers currently found reliable in diagnosing and prognosticating UTI in children include serum

procalcitonin, urine/serum IL-6 and IL-8, urine NGAL, urine IL-1β, and urine 8-oxodG. Serum procalcitonin is particularly useful in predicting UTI complications (such as vesicoureteral reflux) and as a marker of febrile UTI severity, while urine/serum IL-6 and serum procalcitonin can be used to differentiate upper UTI from lower UTI. All studies that investigated these biomarkers established culture-positive UTI, highlighting a direct correlation between positive urine cultures and increased concentrations of the biomarkers in body fluids.

MORE RECENT NOVEL BIOMARKERS IN URINARY TRACT INFECTION DIAGNOSTICS

In the past few years, more novel biomarkers (significantly elevated in culture-proven UTI) are still emerging and are currently being investigated. Given the accumulating evidence on the utility of novel biomarkers in UTI diagnostics, there could be a paradigm shift to incorporate the microbiological evaluation (positive urine cultures) with biochemical evaluation (elevated serum/urine novel biomarkers) if there is a strong correlation between positive urine cultures and raised levels of these novel biomarkers. However, the diagnostic paradigm would have to be validated by scientific data that support this direct association.

In a recent prospective cohort study of children with culture-positive UTI and those with culture-negative pyuria, cathepsin S (CTSS) levels were elevated in the urine of children with the former when compared with the urine of children with the latter.[35] CTSS is a lysosomal enzyme expressed by antigen-presenting cells such as macrophages (mononuclear phagocytes) and B lymphocytes (Figure 1). Immune cells (including macrophages) secrete CTSS in response to inflammatory mediators such as proinflammatory cytokines and neutrophils. In the bacterial infection of the urinary tract, the mononuclear phagocytic system involved in innate immunity produces this novel biomarker (CTSS), which subsequently appears in the urine and could, therefore, be regarded as a surrogate of culture-positive UTI.

The diagnostic performance of urine NGAL in childhood UTI has been previously discussed. Another recent study has underscored its superiority over other biomarkers, including CRP and procalcitonin, in distinguishing between acute bacterial and viral infections.[36] The authors demonstrated human neutrophil lipocalin or NGAL levels in serum or whole blood activated by formyl-methionine-leucine-phenylalanine and showed this discriminating ability with high accuracy. Furthermore, the procedure is suggested as a potentially acceptable point-of-care investigation due to its response time of less than 15 minutes.[36] Thus, a raised serum NGAL in children with UTI predicts a bacterial aetiology and suggests a possible concordance with a positive urine culture. Serum NGAL has been reported as an ideal biomarker for the early diagnosis of culture-proven febrile UTI (acute pyelonephritis), with sensitivity and specificity higher than those of urine NGAL.[37] A recent report suggests that the predictive values and likelihood ratios of urine NGAL were superior to those of serum CRP and white blood cell count for detecting febrile and non-febrile UTI at each cut-off level.[38] However, urine NGAL levels are influenced by the urine sampling technique. Bagged urine samples exhibited lower quantitative and dipstick NGAL specificities than catheterised urine samples.[39] Other studies also established that urine NGAL expression temporally correlates with the bacterial stimulus, responds in a quantitative trend to the bacterial colony count, and rapidly reverses with the resolution of infection.[40-42]

Furthermore, antimicrobial peptides (human α-defensin 5 [HD5] and human neutrophil peptides 1–3 [HNP1–3]) were recently evaluated for their diagnostic accuracy in children with culture-positive UTI.[43] HNP1–3 is a neutrophil-related biomarker and indicates the presence of pyuria. In contrast, HD5 is expressed throughout the urothelium of the lower urinary tract and in the nephron and renal collecting tubules. In order of frequency, the bacterial isolates accounting for positive urine cultures included *E. coli*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, and *Serratia marcescens*. Urinary HD5 and HNP1–3 levels were significantly higher in culture-positive urine samples than culture-negative urine samples. When combined with leukocyte esterase (detected on urinalysis), HD5 significantly increased specificity without decreasing

sensitivity, especially when the urine sample was collected by the clean-catch method.[43] These findings highlight, to some extent, the diagnostic utility of the biomarkers and their dependence on the urine-sampling method.[39] Urine HD5 and NGAL are more likely to correlate with UTI aetiologically attributed to *E. coli* than with UTI from other bacterial agents since pyuria frequently occurs in the former.[22] However, there is no consensus on the diagnostic value of these two biomarkers as they also exhibited poorer diagnostic utility in another study.[44]

In a recent comparative study, 30 children with culture-positive febrile UTI and 30 healthy controls were investigated for the diagnostic value of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) as a novel biomarker for childhood UTI.[45] sTREM-1 had a sensitivity of 57% and a specificity of 50% for the diagnosis of UTI. TREM-1 is a protein surface receptor that amplifies toll-like receptor-induced inflammation by increased production of proinflammatory cytokines.[46] Its soluble form (sTREM-1) accumulates in the bloodstream during inflammation and has been used to provide valuable information on the degree and outcomes of inflammatory processes in sepsis and pneumonia,[47] as well as inflammatory bowel disease.[48] sTREM-1 does not have a high sensitivity and specificity compared with previously established biomarkers like serum procalcitonin and NGAL. However, bacterial infection is an essential factor that increases TREM-1 expression, providing reliable evidence that positive urine culture most likely correlates with this novel biomarker.

Serum calprotectin (sCAL) is another potentially predictive biomarker of bacterial UTI in febrile children.[49] sCAL is a heterodimer consisting of S100A8 and S100A9 proteins from the family of S100 proteins, with suggested clinical value as a biomarker of inflammation. It has now been investigated as a possible biomarker for bacterial UTI in children. Among 22 children with culture-positive UTI, sCAL showed a sensitivity of 93.1% and specificity of 76.2%. Thus, sCAL levels in febrile children could be an additional biomarker of consideration in the prompt and accurate diagnosis of UTI.[49] This lends credence to the role of inflammatory biomarkers as potential predictors and biomarkers of UTI.

Another recent study investigated the serum levels of oxidative stress biomarkers such as malondialdehyde (MDA) and total antioxidant capacity (TAC) in children with culture-positive UTI.[50] Oxidant–antioxidant balance biomarkers (MDA and TAC) were quantified in their serum samples. Urine culture was performed to identify the causative organism of UTI. Overall, a significant elevation in serum MDA and a decrease in serum TAC was found. *E. coli* was the most frequent bacterial aetiologic agent for UTI in these children. It was concluded that the rise in serum MDA and reduction in serum TAC levels also implied an attenuation in the protective effects of antioxidants, given that low levels of reactive oxygen species are typically produced during tissue metabolism.[50]

Table 1 summarises the potential indications of the established and more recent novel biomarkers. Beyond their utility in UTI diagnostics,[53] they have hitherto been found useful in various other diseases.[51,52,54] Obviously, these biomarkers are highly sensitive and specific in detecting bacteria-induced inflammatory diseases.

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Identifying novel biomarkers that are more accurate than conventional biomarkers (e.g., nitrite or leukocyte esterase) may help to enhance UTI diagnosis and minimise inappropriate antibiotic treatment in children with the infection. Thus, novel UTI diagnostics hold prospects as alternative tools for improved management of UTIs. This review has shown that established and more recent serum and urinary biomarkers exhibit disparate predictive and diagnostic abilities for UTI and its related complications. Some have elevated differential levels in upper and lower UTI (or febrile and non-febrile UTI), and thus could be used to distinguish between the two forms of UTI. Their sensitivity and specificity values vary from moderate to high percentages. Most studies reporting their diagnostic value also documented culture-positive UTIs in the evaluated children. Because certain uropathogens are less likely to be associated with pyuria, the sensitivities of some neutrophil-related novel biomarkers (such as urine NGAL and HNP1–3) are reduced in cases of UTI caused by these bacteria. While

Table 1: Potential indications and sensitivity/specificity values of established and more recent novel biomarkers in urinary tract infections.

Novel biomarkers	Evidence-based potential indications	Specificity values (true-negative rates)*	Sensitivity values (true-positive rates)*
Established novel biomarkers			
Procalcitonin	Marker in bacteraemia	70%[51]	76%[51]
	Marker in severe sepsis	N/R	N/R
	Differentiating SIRS from sepsis	91%[52]	90%[52]
	Marker of acute pyelonephritis	89.7%,[25] 89.8%[26]	94.1%,[25] 86.2%[26]
	Differentiating upper UTI from lower UTI	58%[24]	98%[24]
	Predicting UTI complications (e.g., high-grade VUR)	32.0%[30]	94.3%,[11] 90.0%[30]
IL-6 and IL-8	Marker in acute pyelonephritis†	100.0%[53]	58.3%[53]
IL-6	Differentiating upper UTI from lower UTI	N/R	N/R
NGAL	Marker in AKI	N/R	N/R
	Marker in acute pyelonephritis‡	76%[33]	97%[33]
IL-1β	Marker in acute pyelonephritis	N/R	N/R
8-oxodG	Prediction of renal parenchymal injury in UTI	N/R	N/R
More recent novel biomarkers			
Cathepsin S	Surrogate (marker) of culture-proven UTI	N/R	N/R
HD5	Marker in UTI	65%[43]	100%[43]
HNP1–3	Marker in UTI	65%[43]	97%[43]
sTREM-1	Marker in sepsis and pneumonia	N/R	N/R
	Marker in inflammatory bowel disease	N/R	N/R
	Marker in UTI	50%[45]	57%[45]
sCAL	Marker in UTI	76.2%[49]	93.1%[49]
MDA and TAC	Oxidant–antioxidant balance as adjunct in the diagnosis of UTI	N/R	N/R

*Values reported in the referenced studies.

†Serum/urine IL-6 more sensitive and specific than serum/urine IL-8.

‡Serum NGAL more sensitive and specific than urine NGAL.

AKI: acute kidney injury; HD5: human α-defensin 5; HNP1–3: human neutrophil peptides 1–3; MDA: malondialdehyde; NGAL: neutrophil gelatinase-associated lipocalin; N/R: not reported; sCAL: serum calprotectin; SIRS: systemic inflammatory response syndrome; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; TAC: total anti-oxidant capacity; UTI: urinary tract infection VUR: vesicoureteral reflux; 8-oxodG: 8-hydroxy-2′-deoxyguanosine.

levels of these novel biomarkers directly correlate with positive urine cultures, it appears that there is no single biomarker with the optimal sensitivity and specificity. Worse still, the shared pathophysiological mechanism of other bacterial infections in producing biomarkers (such as procalcitonin) makes it challenging to have a specific biomarker for UTI. However, some biomarkers' sensitivity and specificity for UTI are augmented with conventional biomarkers like leukocyte esterase. Although these novel biomarkers are promising, translating their measurements into clinical practice with specific clinical utilities will take time. On the other hand, novel methods interrogating high-throughput serum (and urine) metabolome data with positive urine cultures in a platform-agnostic manner (metabolome-wide approach)

will help confirm and identify novel biomarkers that might capture specific aetiologic agents or shared pathways of related agents. Thus, future research on UTI diagnostics should specifically focus on identifying highly sensitive and specific standalone novel biomarkers that can be easily applied as a point-of-care investigation.

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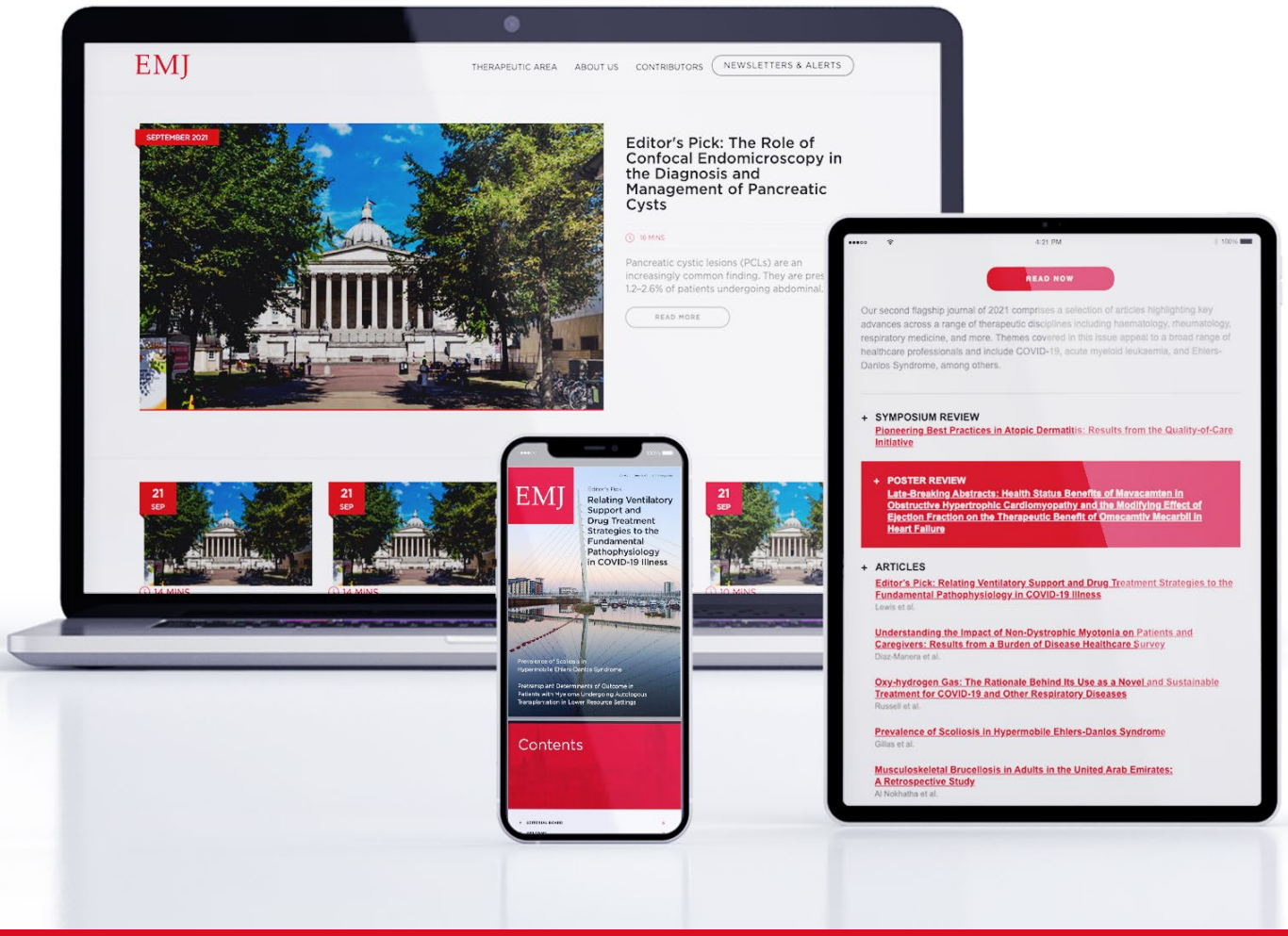

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Aviptadil: Class Effect of a Synthetic Vasoactive Intestinal Peptide as a Treatment Option in Patients with COVID-19 with Severe Respiratory Failure

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Abstract

Despite dynamic drug and vaccine development processes to reduce the disease burden of COVID-19, the treatment options are still very limited. Vasoactive intestinal peptide (VIP) has a diversified physiological action with specific features of lung protection-related activities.

VIP inhibits severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) gene replication in human monocytes and the viral replication in Calu-3 cells, thus further reducing the generation of proinflammatory mediators. Aviptadil, a synthetic form of VIP, is the only pulmonary therapeutic agent to have been granted 'fast track' status by the U.S. Food and Drug Administration (FDA) and to be allowed into both Phase II and III clinical trials. Initial binding of Aviptadil with non-structural protein (nsp) 10 and nsp16, which may inhibit the 2'-O-methyltransferase activity of the SARS-CoV-2 nsp10 and nsp16 complex.

Aviptadil has already proved to be an effective option in the treatment of severe respiratory failures due to sepsis and other related lung injuries. Interim analysis results of this drug used in respiratory failure caused by SARS-CoV-2 has evolved a new hope in regard to safety and efficacy. The final results from a recently completed trial, as well as all currently ongoing trials, will clarify the class effect of this drug in the treatment of COVID-19 in future days.

INTRODUCTION

COVID-19 has created an unprecedented global situation over the last 18 months. As of 23rd November 2021, there have been 257,469,528 confirmed cases of COVID-19 reported to the World Health Organization (WHO), including 5,158,211 deaths worldwide. This pandemic has led to extensive research to evaluate the safety and efficacy of several repurposed but also some new drugs. Numerous clinical trials are ongoing to establish the clinical benefits of these drugs.

The mainstay of the treatment continues to be based on supportive care, with the possible use of pharmacological agents in patients with more severe illnesses. Antiviral agents such as remdesivir may help to shorten the duration of illness but may not be efficacious enough to provide the survival benefit in life-threatening situations. Hypoxic individuals, as well as those requiring supplemental oxygen and non-invasive or invasive ventilatory support, treated with low-dose steroids have shown improved survival outcomes, with robust data supporting the statistically significant clinical outcome parameters.

In an adjunct to that, parenteral as well as oral anticoagulants have shown promising results in regard to combatting fatal complications such as a pulmonary embolism in cases of moderate-to-severe illness, where individuals are hospitalised in dedicated isolation wards as well as intensive care settings. Convalescent plasma has not lived up to the promise it held as the recent randomised clinical trials failed to demonstrate any added benefit as far as the mortality and morbidities of the disease are concerned. Cytokine inhibitors such as tocilizumab and other immunomodulatory drugs need further evaluation among a larger participating population of clinical trials to establish their efficacy.[1]

After performing an extensive literature review using three databases (PubMed, Scopus, and Cochrane), it has been found that, in spite of dynamic drug and vaccine development processes to reduce the disease burden of COVID-19, the disease may not be eradicated due to the evolution of newer mutant strains of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and logistical challenges in the administration of vaccines globally. Due to these reasons, the cornerstone of such disease management remains dependent, to a certain extent, on novel drug discoveries and their accelerated regulatory approval to be used on basis of investigational therapeutic tools.[2]

Vasoactive intestinal peptide (VIP) was first isolated from a hog's intestine by Said and Mutt in 1970.[3] It is mainly located in the myenteric and sub-mucosal neurons as well as nerve terminals of the gastrointestinal tract and contains 28 residue amino acid peptides. Apart from the digestive system, it is widely distributed in both the peripheral and central nervous systems as well as the cardiovascular, respiratory, and reproductive systems. This gut peptide hormone belongs to the glucagon/secretin hormone superfamily and is produced by neuroendocrine cells, macrophages, and both B and T lymphocytes.[4,5]

PHYSIOLOGY OF VASOACTIVE INTESTINAL PEPTIDES

VIP is highly expressed in the lung tissue (approximately 70%) and nasal mucosa.[6] It exerts its action in the lung tissue via two types of receptors acting as G protein-couple receptors; VIP receptor Types 1 and 2 (VPAC1 and 2); and receptors that are also activated by the pituitary adenylate cyclase activating polypeptide, which belongs to the same family as VIP. The VPAC1 receptor is predominantly located in lung tissue and T lymphocytes whereas VPAC2 is found on smooth muscle, mast cell, and basal part of lung mucosa.[7] VIP binds to alveolar Type II (AT-II) cells via the VPAC1 receptor.[8] Despite comprising of only 5% of the total lung tissue, it plays an important role in surfactant production, which helps in the maintenance of alveolar Type I epithelial cells.

VIP augments surfactant production by upregulating the enzyme choline phosphate cytidylyl transferase, which induces the incorporation of methyl choline to phosphatidylcholine, a major component of pulmonary surfactant.[9] Additionally, it induces the c-Fos protein expression in AT-II cells and upregulates surfactant protein A expression, both of which ultimately lead to surfactant production.[10] It also inhibits apoptosis by blocking the activities of caspase, granzyme B, and perforin.[11,12] It exerts non-adrenergic, non-cholinergic bronchodilatation, which is 100 times more potent than isoproterenol and 50 times more potent vasodilatation in both systemic and pulmonary arteries than prostacyclin.[13,14] Preclinical experiments in mouse models have demonstrated their role in reducing ischaemia-induced reperfusion injuries.[15]

Besides the impact on respiratory physiology, VIP also has several other significant actions such as:

- Positive chronotropic, ionotropic, and coronary vasodilatory actions

- Secretion of water and electrolyte on gastrointestinal lumen, pancreatic juice, and bile
- Stimulation of pepsinogen secretion
- Regulation of Prolactin secretion and promoting vaginal lubrication
- Inhibiting T lymphocyte proliferation[16]
- Promoting T-helper 2 lymphocytes differentiation against T-helper lymphocytes and regulatory T cell induction[16,17]
- Downregulating several macrophage-mediated inflammatory cytokines and proinflammatory receptors[18]
- Playing a role of an inhibitory neurotransmitter of the non-adrenergic, non-cholinergic autonomic nervous system[19]
- Inhibiting the synthesis as well as activation of nuclear factor κ-light-chain-enhancer of activated B cells, which block the process of TNF-α generation[20]

RATIONALITY BEHIND OF USE OF VASOACTIVE INTESTINAL PEPTIDES IN COVID-19

Acute respiratory failure is a major cause of death due to the SARS-CoV-2 infection. It is attributed to a cytokine storm, preceded by the invasion of the alveolar cell by the virus itself and then rupture of that pulmonary epithelial cell. This invasion occurs once the virus enters the AT-II cell by binding its spike protein to the surface of the angiotensin-converting enzyme 2 receptors located on AT-II cells.[21] AT-II cells express VPAC1 receptors on its surfaces, which the VIP binds to and prevents the process of apoptosis in lung injury.[22] VIP inhibits SARS-CoV-2 gene replication in human monocytes and viral replication in Calu-3 cells, thus further reducing the generation of proinflammatory mediators that play a significant role in tissue injury in the course of the COVID-19 disease.[23]

VIP has demonstrated its beneficial effects on lung injuries in several animal models (Table 1). Unlike the anti-IL-6 drugs, it preserves the surfactant production as well as protects the AT-II cells of the lung.[4,23,28] Other than the surfactant producing and anti-inflammatory activity, VIP has the property of potentially inhibiting the Fas ligand expression and thereby halting the progression of Fas ligand-mediated cell death.[9,17] Acute lung injury caused by the COVID-19 is also contributed to by the degranulation of serine proteases granzymes and the formation of perforin protein, which induces the rapid death of the target cells.[21] As a proven inhibitor of granzyme and perforin, VIP plays an important role in the prevention of cell death in lung tissue.[29]

AVIPTADIL USE IN CLINICAL PRACTICE

Aviptadil is a synthetic form of VIP, which is also known as RLF-100. It has been designated as an orphan drug by the U.S. Food and Drug Administration (FDA) to treat respiratory airway diseases such as asthma, chronic obstructive airway disease, cystic fibrosis, pulmonary hypertension, adult respiratory distress syndrome (ARDS), lung fibrosis, and sarcoidosis as well as in non-respiratory conditions such as erectile dysfunction.[11]

It is available both intravenously and as inhalational preparations. The half-life of the drug is 1–2 min and the apparent volume of distribution is 14 mL/kg. This drug is almost eliminated by the renal route, where 35% elimination occurs in the first 4 hours and 90% occurs within 24 hours. It has no significant clinical drug–drug interactions and insufficient data is available on its use in pregnancy and lactation. Intravenous administration is associated with side effects like tachycardia, flushing, hypotension, diarrhoea, and alterations in ECG (bigeminy).[30]

Patients with pulmonary arterial hypertension were successfully treated with inhaled aviptadil, which caused a reduction in pulmonary artery pressure and improved in cardiac output and mixed venous oxygen concentration.[31] In another open label Phase II clinical study, 20 patients with histologically proven sarcoidosis were treated with inhaled aviptadil for 4 weeks, which caused a significant reduction of TNF-α and the increment of CD4+ CD127− CD25+ T cells in their bronchoalveolar lavage fluid.[32]

A safety evaluation of aviptadil was performed by conducting five Phase II trials under the observation the European Medicines Agency (EMA) and aviptadil was found to be a well-tolerated drug, with fewer side effects such as hypotension, flushing, and diarrhoea. Another open label Phase I study was conducted in 2005

with 8 patients suffering from sepsis-related ARDS (all were on mechanical ventilation), who had been treated with an intravenous aviptadil infusion over 12 hours (50–100 pmol/kg/hour). Seven among those eight critically ill patients were successfully taken off of the ventilator and discharged home. Other than that, no drug-related serious adverse events were recorded and serial estimation of the serum blood TNF-α level showed significant decrement at the end of the treatment.[33]

ROLE PLAYED BY AVIPTADIL IN COVID-19

SARS-CoV-2 infection is characterised by a hyperimmune response and dysregulated productions of cytokines and chemokines, which play a pivotal role in severe lung injury and unfavourable clinical outcomes in patients with COVID-19.[34-37]

Several non-structural proteins (nsp) play a significant role in SARS-CoV-2 viral RNA replication process. Among them, the SARS-CoV-2 nsp16–nsp10 complex works as a 2'-O-methyltransferase.[38] This complex is also necessary to evade the immune recognition process.[39] Results of *in silico* structural bioinformatics analyses have demonstrated the potential sites of binding specificity between aviptadil and nsp16. The interaction model also showed the process of the initial binding of aviptadil with nsp10 and nsp16, which may inhibit the 2'-O-methyltransferase activity of the SARS-CoV-2 nsp10/16 complex.[40] The SARS-CoV-2 virus enters the AT-II cell through the binding of its spike protein to the angiotensin-converting enzyme 2 surface receptors.[41] Unlike the AT-I cells, only AT-II cells express the VPAC1 receptor that VIP binds to, thus VIP and its analogues deserve special attention as a therapeutic option to combat the hypoxemic lung injury in COVID-19.

Aviptadil is the only pulmonary therapeutic agent to have been granted 'fast track' status by the FDA and to be allowed into both Phase II and III clinical trials, as well as an expanded use protocol for those who are unable to enter the clinical trial due to excluded comorbidity.

The initial use of aviptadil via an intravenous route for the first time (after getting the authorisation of emergency use as an investigational new drug from the FDA) was reported in a case report by Jihad Georges Youssef et al. when a double lung transplant patient (additionally in a stage of antibody-mediated rejection) got infected with SARS-CoV-2 and subsequently developed severe respiratory failure and was treated with aviptadil at Houston Methodist Hospital, Texas, USA. After the third dose of aviptadil via infusion, there was a dramatic improvement in oxygen saturation and radiographic changes, which ultimately lead to the patient being discharged from the hospital and was alive until 28 days after post-discharge, as per the latest information gathered from the study.[22]

In another case series, 21 consecutive lab-confirmed patients with SARS-CoV-2 and multiple comorbidities showed significant improvement both from the radiological as well as a clinical point of view after being treated with intravenous aviptadil. Most were sent back home after being weaned frommechanical ventilation and decannulated from extracorporeal membrane oxygenation support, which was also associated with biochemical improvements in the form of the steady decline of inflammatory markers (e.g., IL-6 and C-reactive protein).[42]

Currently, nine clinical trials are on the list (two of which are in India), where aviptadil is being tried via both the inhalational and intravenous route and subsequently tested based on some outcome parameters to assess the safety and efficacy of the drug in comparison to the use of placebo, remdesivir, monoclonal antibodies, and other immunosuppressants in the treatment of COVID-19 that is complicated by severe respiratory failure. The details of those trials are summarised in Table 2. Among those nine trials, seven are in the recruitment stage, comprising the trial with maximum sample size, where one trial is completed and the data of the remaining one is available recently.

Of all the trials mentioned in Table 2, only one trial has been completed recently (in February 2021), which demonstrated promising results on the intravenous use of aviptadil in COVID-19. In this multicentre, placebo-controlled trial, 196 patients with COVID-19 respiratory failure were randomised 2:1 to receive 3 days of intravenous aviptadil or placebo. The primary endpoint was "alive and free from respiratory failure at

Table 1: Effect of vasoactive intestinal peptides on various experimental animal models of acute lung injury.

Animal models tested for	Aetiopathology of lung injury	References
Rat	NDMD induced lung injury with arginine	Said (1996); Dickman et al. (2000)[24]
Guinea pig	Paraquat (methyl viologen)	Pakbaz et al. (1993)[25]
Rat	Hydrochloric acid induced pulmonary oedema	Foda (1988)[26]
Rat	Cobra venom factor model of septic shock	Mulligan (1992)[27]

NDMD: N-dimethyl daunomycin.

Table 2: Summary of current trials of aviptadil in COVID-19.

Trial identifier	Drugs used	Location	Recruitment status	Estimated sample size
NCT04844580[43]	Aviptadil; placebo	Turkey	Recruiting	80
NCT04536350[44]	Aviptadil; placebo	Switzerland	Recruiting	82
NCT04360096[45]	Aviptadil; placebo	USA	Recruiting	144
NCT04843761[46]	Aviptadil; remdesivir; placebo	USA	Recruiting	640
NCT04488081[47]	Aviptadil; remdesivir; pulmozyme; celecoxib; famotidine; narsoplimab; yclosporine; IC14	USA	Recruiting	1,500
NCT04311697[48]	Aviptadil; placebo	USA	Completed	196
NCT04453839[49]	Aviptadil	USA	Available	196
CTRI/2021/04/033118[50]	Aviptadil; placebo	India	Recruiting	150
CTRI/2021/06/034373[51]	Aviptadil; placebo	India	Recruiting	152

day 60." The investigators also studied the mechanistic effect of aviptadil on blocking cytokine production and its link to survival and recovery from respiratory failure. It was found that, when controlling for baseline severity and site of care, patients treated with aviptadil were significantly more likely to be alive and free from respiratory failure at 60 days compared with those treated with placebo (p=0.02) and demonstrated significance on numerous other clinical endpoints. Without controlling for the site of care, a two-fold increased odds of survival was seen at 60 days (95% confidence interval: 1.0–3.9; p=0.035). Biomarker analysis demonstrates that aviptadil significantly decreased the probability of an IL-6 increase relative to placebo (50% versus 71%; p=0.04) and that preventing this cytokine rise was highly correlated with survival

and recovery ($p<0.0001$), regardless of baseline severity or treatment site.[52]

As the study is limited by its insufficient power and other important outcome analysis as well as the 60-day endpoint assessment as reports are still awaited, it is too early to conclude the study objective of this trial. It is also true that, as per the available data about the use of this molecule in ARDS-related sepsis as well as its use in the preclinical lung injury model, this molecule retains the hope to become an effective treatment option in the management of respiratory failure caused by SARS-CoV-2 infection.

CONCLUSION

Aviptadil, as a synthetic VIP, holds a promising place in the armamentarium of treatment of SARS-CoV-2. The class effect of this drug is already established in the almost similar clinical scenario of ARDS caused by sepsis as well as other related lung injuries in preclinical models. The safety data of this molecule has also had a favourable notation on the use in several respiratory airway diseases. Current interim analysis data about the safety and efficacy of this molecule is encouraging in spite of limitations regarding the sample size of the study affecting the power. Upcoming results from the ongoing clinical trials will play a pivotal role in treatment policy-making aspects. More robust data on a larger target population will be immensely helpful to prove its impact on the reduction of the disease burden in the treatment of this deadly virus.

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Zika Virus Infection and Pathogenesis

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Abstract

Zika virus (ZIKV) is a flavivirus that was met with relatively little acclaim when it was discovered in 1947. Initial clinical reports of ZIKV included asymptomatic infection or mild, febrile illness; however, the view of ZIKV as an insignificant virus changed dramatically following the epidemic in the Western Hemisphere that started in 2015. This epidemic featured central nervous system involvement in children and adults, and a devastating congenital syndrome following infection of pregnant women. While the pathogenicity of ZIKV was virtually undescribed prior to this epidemic, in the past few years, numerous reports have described receptor–ligand interactions, aspects of tissue tropism, host–pathogen interactions, and diversity across viral clades. In this paper, the variety of clinical presentations and virulence determinants of ZIKV are reviewed.

HISTORY AND EPIDEMIOLOGY

Zika virus (ZIKV) was first isolated in 1947 from rhesus monkeys during a routine surveillance of yellow fever in the Zika Forest of Uganda. A year later, ZIKV was recovered from the mosquito species *Aedes africanus* in the same area.[1,2] By 1952, the first human cases had been detected in the United Republic of Tanzania, Uganda, and India via antibody neutralisation tests. Throughout the 1950s, human serology detection of ZIKV in adults and children arose in Nigeria, the British colonies of Malaya and North Borneo (both currently part of Malaysia), the Philippines, Nigeria, Egypt, Vietnam, and Mozambique.[3] In 1958, two additional strains were isolated from *A. africanus* species that were collected in the Zika Forest.[4] The first report of human illness caused by ZIKV came in 1964 and consisted of a mild febrile state and maculopapular rash, thereby confirming that ZIKV is a causative agent of human disease.[1,3,5]

From 1960 through to the early 2000s, ZIKV was sporadically detected in humans via haemagglutination inhibition and other serological methods.[3,6] Symptomatic cases were rare, leading to the relatively benign designation of ZIKV disease. ZIKV was continuously isolated from sentinel rhesus monkeys that were used for field research, as well as from numerous

mosquito species, predominantly of the genus *Aedes*, in several African countries. By the end of the 20th century, the geographical distribution of ZIKV expanded throughout equatorial Asia, with confirmed widespread population exposure in Indonesia, Malaysia, and Pakistan. This included ZIKV detection in mosquitoes and sporadic human cases, but no epidemic disease.[3]

The first widespread epidemic associated with ZIKV occurred on the Pacific island of Yap in 2007.[7,8] During this time, 185 suspected cases of ZIKV infections were recorded, 49 of which were confirmed via PCR and 59 of which were presumed probable in patients with the IgM antibody against ZIKV; no deaths were recorded.[3] The introduction of ZIKV to the Yap population was suspected to be caused by travel and trade, either through infected humans or mosquitoes. This theory was reinforced by the lack of monkeys present on the island that could act as sentinel reservoirs, as well as the publication of two geographically distinct ZIKV lineages in 2012, each with multiple strains. Nucleotide sequencing of ZIKV isolates from multiple countries provided strong evidence that the ZIKV strains responsible for the epidemic in Yap emerged from Southeast Asia.[3,9]

ZIKV outbreaks in the Pacific islands continued to occur throughout the years that followed.[3] In March 2015, the largest and most recent ZIKV epidemic began in Brazil. The rapid spread of infection through *Aedes* mosquitoes and sexual transmission led to Brazil declaring a national public health emergency in November 2015. ZIKV then continued to rapidly spread throughout the Americas, prompting the World Health Organization (WHO) to declare a Public Health Emergency of International Concern in February 2016.[10] This ZIKV epidemic was associated with many new symptoms and lasting effects, provoking intense investigations. The reports that followed marked the associations of ZIKV with congenital syndromes, malformations, meningoencephalitis, and Guillain–Barré syndrome.[3] Retrospective analyses indicated that these syndromes were also present in the South Pacific in the preceding years, although strong associations were not made at the time.[11] In the spring of 2016, the European Union (EU) and the USA strongly advised that women in any stage of pregnancy should postpone travel to countries with known local transmission of ZIKV. There is currently no vaccine or treatment for ZIKV, so it remains an ongoing concern and a potential threat to public health.[10]

ZIKA VIRUS BIOLOGY, TRANSMISSION, AND CLINICAL PRESENTATIONS

ZIKV belongs to the genus *Flavivirus*, a taxon of single-stranded, enveloped RNA viruses that also includes dengue virus (DENV), yellow fever virus, West Nile virus (WNV), Powassan virus, and many others.[12] The transmission route of flaviviruses is primarily vector-borne, resulting in a wide range of clinical diseases.[12,13] ZIKV is primarily transmitted by *Aedes* species mosquitoes, specifically *Aedes aegypti* and *Aedes albopictus*.[13,14] Additional routes of flavivirus transmission include horizontally from mother to fetus, via blood or issued products containing the virus, and, for certain species such as ZIKV, sexual transmission.[13] Once inside the human host, flaviviruses are able to enter host cells through receptor-mediated endocytosis and utilise the cells' resources in order to replicate and promote further infection.[15]

Clinical disease produced by ZIKV and related flaviviruses varies from asymptomatic or mild symptoms to severe nervous system deficits and fetal deformities. Mild symptoms include fever, rash, headache, arthralgia, myalgia, conjunctivitis, and uveitis.[14,16] Flaviviruses are a neuroinvasive group, and infections can potentially culminate in severe neurological diseases, including encephalitis, meningitis, and seizures.[16,17] Pregnant women have a risk of transmitting the virus to the fetus, resulting in adverse pregnancy outcomes for some viruses and congenital Zika syndrome for cases of ZIKV. The effects of ZIKV on a developing fetus have been found to focus around the nervous, musculoskeletal, and visual body systems, though effects can be found throughout the body.[18] The most severe presentation of congenital Zika syndrome in liveborn infants is microcephaly.[19] Other notable neurologic manifestations include ventriculomegaly, hydrocephalus, and hypoplasia or atrophy of the cerebral cortex, cerebellum, and brainstem.[18] Congenital Zika syndrome has also presented with clubfoot, patent foramen ovale, dysphagia, and hearing and vision loss.[19] The disease presentation of ZIKV infection correlates both with the predominant cell targets and the

elicited immune responses of the host, making host cell binding and host–pathogen interactions critical factors.[3,20]

ASPECTS OF ZIKA VIRUS VIRULENCE

Host Cell Binding

The ZIKV genome encodes three structural proteins (capsid; precursor membrane [prM]; and envelope [E]), seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5), and two non-coding regions at the 3'- and 5'-terminal ends.[21] Host cell adsorption is mediated by the E protein. After infection, the E protein mediates the binding of ZIKV to host cell entry factors and surface receptors, before undergoing clathrin-dependent endocytosis.[21] A specific binding motif (E-N-R-A-K-V, E protein amino acid positions 162–167) both directly binds to ZIKV-permissive cell lines and significantly inhibits ZIKV adsorption *in vitro* by competing for the same receptor, strongly implicating this portion of the E protein as a functional driver of host cell binding. This motif is notably proximal to the asparagine at position 154, which is implicated in host cell interactions by the four DENV serotypes (DENV-1–4), and in the neuroinvasion of WNV and St. Louis encephalitis virus.[22-24] Flaviviruses use a variety of receptors to mediate entry into host cells, making it difficult to establish targets for the prevention of ZIKV infection.[25] ZIKV entry receptors include AXL, DC-SIGN, TYRO3, and TIM-2 among others. These receptors are present in cells of the brain, skin, testes, placenta, kidneys, retina, and immune system, with ZIKV favourably targeting primary human brain microvascular endothelial cells. The first described receptor was AXL; however, genetically ablated animals were still susceptible to infection, which indicated that multiple viral entry mechanisms must exist.[21,25,26]

Once infection is established in mammalian host cells, antiviral responses in the host are stimulated, leading to the activation of inflammation as well as humoral and innate immune responses. Inflammation is mediated by cytotoxic (CD8+) T cell activation, leading to downstream cytokine and chemokine release. The humoral immune response includes the production of IgG and IgM protective antibodies against ZIKV, and the innate immune response is responsible for the recognition of ZIKV and activation of antiviral responses.[25] In order to bypass these host immune responses, ZIKV has established numerous mechanisms of evading destruction. For example, once ZIKV infection is established, it blocks Type I and Type III interferon (IFN) induction, which hampers the innate immune responses of the host and establishes resistance to IFN treatment. ZIKV also mediates the inhibition of the JAK-signal transducers and activators of transcription signalling pathway and IFN-β production, and also induces cytopathic effects and apoptosis in human neural progenitor cells (hNPCs).[25] The infectious outcome of ZIKV is dependent on the balance between the antiviral immune responses of the host and the counteracting mechanisms of ZIKV.[25]

Variations in Pathogenesis Across Zika Virus Lineages

There are two major phylogenetic lineages of ZIKV: the Asian and Western Hemisphere clade and the African clade. All ZIKV human epidemics to date have been caused by strains belonging to the Asian lineage, and only Asian clade strains have been linked to congenital abnormalities and neurological disorders.[27] The African clade strains have a higher transmissibility in *A. aegypti* mosquitoes; however, they also exhibit a lower replication efficacy in vertebrate cells compared to that of Asian strains, which may be a contributing factor in the reduced pathogenicity of the African strain in humans.[27,28]

The region of highest genetic variability amongst the African and Asian strains occurs in the 'pr' peptide portion of the prM protein.[29] The functional impact of this variability is widespread, and the evolutionary drivers are likely a combination of immune selection and changes in binding avidity and/or specificity. One position within the pr region with multiple known amino acid changes is position 17. The consensus sequence from African lineage strains show a serine at position 17, whereas Asian lineage strains have modified this to an asparagine. This change was strongly associated with increased neurovirulence and microcephaly in a fetal mouse model, likely by enhancing viral maturation kinetics during infection.[30]

Of the genetic differences that are conserved across strains of Asian and African lineages,

the most extensively studied is the introduction of an N-glycosylation motif (sequence: N-X-S/T) at codons 154–156 of the E protein in the Asian lineage.[28] Changes in binding affinity or avidity and host cell tropism, most notably neuroinvasiveness, have been ascribed to the addition of an N-acetylglucosamine to the asparagine at position 154 in ZIKV and other neurotropic viruses.[23,24,26] In addition, the phosphatidylserine-binding protein annexin V was shown to competitively inhibit the infection of Vero cells by the Asian lineage strain PRVABC59, but did not affect the infection of the African lineage strain MR-766. This suggests that Asian lineage ZIKV can initiate host cell entry via phosphatidylserine-mediated adsorption. This is likely to occur via phosphatidylserine binding to the growth arrest-specific 6 (Gas6) protein, which in turn binds AXL, a tyrosine kinase receptor.[26] AXL downregulates the IFN signalling response, thereby accelerating infection. After binding to AXL, ZIKV enters the cell via clathrin-mediated endocytosis, and thereafter transfers to the Ras analog in brain 5-positive endosomes to establish infection.[31] This facilitates migration to the lymph nodes and then the bloodstream.[11] Compared with other flaviviruses, the Asian lineage ZIKV strain FSS13025 infects fetal endothelial cells more efficiently as it has a higher affinity to bind Gas6, which in turn aids in its interaction with AXL.[32] Taken together, the addition of an N-acetylglucosamine proximal to the E protein binding motif, the ability to enter host cells via phosphatidylserine-driven mechanisms, and the enhanced replication kinetics conferred by alterations in the prM protein plausibly explain the profound differences between the clinical presentations of the African and Asian lineage ZIKV strains.

Antibody-Dependent Enhancement

Antibody-dependent enhancement (ADE) is the phenomenon of a virus stimulating non-neutralising antibodies in order to facilitate its entry into host cells by opsonisation, a process that involves the binding of antibodies to infectious viruses.[33] The non-neutralising antibodies required for this process do not necessarily have to derive from the virus or the strain utilising it, as long as the viral antigen expresses cross-reactivity. ADE has been described in detail for other flaviviruses, most notably DENV-1–4.[34] Individuals infected with DENV generate antibodies during their primary infection, and non-neutralising antibodies facilitate a cell entry that is far more efficient upon reinfection. These enhanced replication dynamics lead to more severe disease, often seen clinically as a haemorrhagic fever as opposed to a simple viraemic fever.[34]

The occurrence of ADE during ZIKV infection is a subject of debate. ZIKV and DENV share numerous antigens, and antibodies from convalescent patients with DENV have been shown to bind ZIKV *in vitro*.[35-40] However, whether a previous DENV infection can lead to enhanced ZIKV disease still appears somewhat ambiguous. At least one study using a rhesus macaque model demonstrated enhanced disease at the maternal–fetal interface in monkeys exposed to DENV prior to infection with ZIKV,[41] and a lethal-challenge model showed elevated ZIKV titres and increased levels of proinflammatory cytokines in mice that received anti-ZIKV antibody infusions prior to the experimental infection.[42] Antibodies against WNV enhanced the cellular uptake of ZIKV, and hyperimmune sera from a large clinical trial of the attenuated DENV vaccine Dengvaxia® were similarly shown to enhance ZIKV entry and replication *in vitro*.[43,44] Despite these persuasive findings, a correlated increase in clinical severity during ZIKV infection post-DENV in humans remains unclear.[45,46] Multiple cases have been reported wherein antibodies from prior ZIKV infections led to enhanced, haemorrhagic disease upon infection with DENV, indicating that prior ZIKV infection can act analogously to prior DENV infection and lead to dengue haemorrhagic fever (DHF).[47] Animal models that have utilised mice and macaques as test subjects are highly consistent with these clinical reports.[48-50] Given the co-circulation of ZIKV and DENV in many parts of the world, the predisposition of convalescent patients with ZIKV to develop the higher-mortality DHF presentation upon infection with DENV is a matter of significant public health concern.

Pathologic Host–Pathogen Interactions

The majority of non-congenital ZIKV infections are asymptomatic or associated with only mild symptoms. In contrast, fetal exposure to ZIKV during the first trimester of pregnancy can present with central nervous system symptoms, including

microcephaly and cortical malformations such as simplified gyral pattern, frontal lobe involvement, and ventriculomegaly.[51] The variety of clinical outcomes indicate a diversity in host–pathogen interactions across tissues, age ranges, and developmental stages. Skin fibroblasts, keratinocytes, and immature dendritic cells are all permissive to ZIKV infection and are the first cells encountered following inoculation via mosquito bite.[52] Host cell entry is mediated by multiple mechanisms as described above, but the application of AXL-mediated entry has notable implications both for inflammatory responses and for the fetus during infection. During mosquito-derived infection, AXL downregulates the IFN signalling response, thereby hampering early antiviral responses and facilitating infection. AXL also plays a role in the ability of ZIKV to cause the congenital disease that is distinctive among flaviviruses. ZIKV displays a higher affinity to bind the AXL ligand Gas6 than other flaviviruses, which gives it a greater capacity to infect placental and fetal endothelial cells.[32] Once the placental barrier has been breached by ZIKV, all host cell entry mechanisms can then be utilised to cause systemic fetal disease.

The mild, usually self-limiting symptoms of ZIKV infection indicate that the innate immune response plays a critical role in controlling ZIKV infections. One of the initial innate immune defences that has been implicated in the response to ZIKV infection is autophagy, which has been shown to increase during infection both *in vitro* and *in vivo*.[53] Melo et al.[53] reported that the vasodilatory peptides angiotensin-(1–7), which are downstream markers of induced autophagy, were increased in the serum of ZIKV-infected patients relative to healthy controls.[53,54] Due to the intricate, reciprocal regulation of inflammation and autophagy, it is tempting to speculate that the observed increase in autophagy is not necessarily unique to ZIKV, but rather a generic indicator of infection. However, stimulating autophagy by the inhibition of modified nucleoside transport resulted in a concomitant increase in ZIKV replication kinetics *in vitro*. This relationship persisted across eight cell lines in the absence of exogenous inflammatory signals, suggesting a strong functional association between autophagy and ZIKV replication.[55] This association is also likely to be a contributing factor to congenital Zika syndrome, as several proteins critical to autophagy also mediate centrosome function.[11]

A major protective component of the innate immune response during ZIKV infection is the Type I IFN (IFN-α and IFN-β) system.[56] On activation, the induction of IFN regulatory factors and nuclear factor-κB occurs, which in turn induces other inflammatory cytokines and chemokines.[57] The ZIKV-induced expression of hundreds of IFN-stimulated genes (ISGs) affects the viral life cycle and viral replication due to their role in RNA processing. While the downregulation of IFN signalling early in infection has been observed *in vitro*, the ultimate expression of ISGs and mild clinical presentation indicate that infected individuals ultimately overcome the initial ZIKV-mediated inhibition. The activities encoded by ISGs in antigen-presenting cells (i.e., dendritic cells and macrophages) are important for T and B cell activation and the development of the adaptive immune response and subsequent virus clearance.[57,58] As dendritic cells and macrophages are preferred host cells for ZIKV during primary infection, the induction of Type I IFNs are critically important for both acute viral clearance and the generation of memory response. However, the stimulation of IFN expression leads to the upregulation of major histocompatibility complex (MHC) class I molecules during flavivirus infections, including ZIKV.[59,60] Increased MHC class I expression during ZIKV infection is followed by increased T cell lysis and the inhibition of natural killer (NK) cell activity.[61,62] Consistent with this, Glasner et al.[60] demonstrated that ZIKV infection went largely undetected by NK cells; therefore, by upregulating MHC class I molecules, ZIKV avoided early detection by NK cells and replicated quickly before T cell responses could be mounted.[60] Ultimately, however, the Type I IFN responses lead to the successful clearance of ZIKV in uncomplicated cases, largely due to the protective role of CD8+ cells.[63,64]

TISSUE-SPECIFIC FINDINGS

Nervous System

ZIKV has been associated with neuroinflammation in children and adults, resulting in meningitis, meningoencephalitis, and an increased number of Guillain–Barré syndrome cases.[11] Fetal microcephaly cases also saw a marked increase

due to maternal infection with ZIKV during the first trimester. Chimelli et al.[65] conducted an analysis of post-mortem infants with confirmed ZIKV infection during the first trimester, identifying ventriculomegaly due to damage to the midbrain with aqueduct stenosis or distortion, as well as small brains with ex-vacuo ventriculomegaly.[65] Well-formed brains with mild calcification were seen in infants where maternal infection occurred later. They also observed an absence of descending fibres consistent with spinal motor cell loss presenting as intrauterine akinesia, arthrogryposis, and neurogenic muscle atrophy. Altogether, these findings suggest that the central nervous system is vulnerable to ZIKV infection during early development.[65]

Absent or decreased Type I IFN responses early in infection amplify ZIKV replication, and central nervous system cells, specifically axons and myelinating oligodendrocytes, have an increased susceptibility when compared with peripheral nervous system cells.[66] ZIKV affects the central nervous system by directly infecting hNPCs that originate from pluripotent stem cells, causing hNPCs to release infectious ZIKV particles, and reducing hNPC numbers by decreasing cell growth, increasing cell death, and causing the dysregulation of cell cycle progression.[67] ZIKV affects haematopoietic cells with microglia, the innate macrophage population localised throughout the brain, and induces a proinflammatory state indicated by elevated immune mediators, such as IL-6, TNF-α, IL-1β, and monocyte chemotactic protein 1.[68] In targeting central nervous system cells, especially human brain cells, ZIKV reduces their viability and growth, thereby reversing neurogenesis during human brain development.[69,70]

Testes

Detection of viable ZIKV in semen, the demonstration of sexual transmission, and clinical reports of haematospermia all clearly indicate that ZIKV expresses gonadal tropism.[11,71,72] Convalescent patients exhibited lower sperm counts and increased sperm abnormalities that persisted for at least 3 months.[73] Insights into testicular pathophysiology and the mechanisms of infertility have been gained from animal models. A 2016 study by Ma et al.[74] that examined ZIKV infection and male infertility in a murine model demonstrated that ZIKV infection can result in the production of pro-inflammatory cytokines and chemokines. These effects were most notable in the testes and epididymis, but not in the prostate or seminal vesicles. The study specifically identified that stem-like testicular peritubular myoid cells and spermatogonia are particularly vulnerable to ZIKV infection, and, in some cases, this can lead to infertility.[74] Another study indicated that ZIKV primarily infected spermatogonia, primary spermatocytes, and Sertoli cells, which caused the destruction of the seminiferous tubules and led to cell death.[75]

Ocular Tissue

ZIKV can involve the eye during mosquito-transmitted or vertically acquired infections. In infected children and adults, ZIKV can cause primary conjunctivitis and uveitis, which are usually self-limiting.[76] In contrast, permanent ocular abnormalities have been detected in many confirmed cases of congenital Zika syndrome. The most common isolated and combined fetal fundus presentations included macular chorioretinal atrophy, chorioretinal atrophy elsewhere, focal pigmentary changes in the macular region, and optic nerve abnormalities.[77] A small 2016 report described three infants with congenital Zika syndrome who had unilateral ocular abnormalities indicating gross macular pigment mottling and foveal reflex loss.[78] A larger study in 2016 identified normal anterior segments in the infants examined; however, there were further occurrences of macular pigment mottling and/or chorioretinal atrophy, as well as optic nerve abnormalities such as optic disc hypoplasia, pallor, and/or an increased cup-to-disc ratio. One infant also presented with horizontal nystagmus.[79] Another 2016 study with a different cohort of patients showed similar findings; however one infant had bilateral iris coloboma and lens subluxation in one eye, indicating an anterior segment finding.[80]

Placenta

Placental damage due to ZIKV infection is likely multifaceted. ZIKV entry via AXL and Gas6 and subsequent lytic replication can cause the necrotic cell death of both trophoblasts and fetal endothelial cells, ultimately compromising the integrity of the placenta.[32] In addition, ZIKV infection of the placenta drives altered lipid metabolism pathways. The placenta has a high

lipid content, and the metabolism of lipids supports fetal development. Disruption of the placental lipid metabolism has been shown to play a role in spontaneous pregnancy loss, intrauterine growth restriction, and other adverse pregnancy outcomes.[81,82] ZIKV infection during pregnancy leads to the reprogramming of the placental lipidome to a profile favourable to viral replication, mitochondrial dysfunction, and a dysregulated inflammatory response.[83]

CONCLUSION

Though ZIKV was not a new virus when the epidemic emerged in the Americas in 2015, it clearly presented with an emerging neurologic and teratogenic pathology. Retrospectively, it is clear that the Asian lineage strains acquired capacities to more efficiently cross the blood–brain, blood–testis, and placental barriers relative to the ancestral African lineage. In this way, ZIKV has gone from a virus causing a benign, self-limiting illness to a virus capable of causing lethal disease in adults, adverse pregnancy outcomes, and/or a severe congenital syndrome. The opinion of the authors is that alterations in tissue tropism and infectivity associated with novel binding partners or altered binding affinity likely led to these new clinical manifestations. Pathologic host responses following the manipulation of the immune response by ZIKV have also been described, and include the modulation of IFN responses and autophagy. Additionally, there is mounting evidence that previous exposure to ZIKV creates the potential for DHF in patients on exposure to DENV. The previously undescribed clinical presentations associated with Asian lineage ZIKV strains illustrate the potential for novel epidemic disease events that are associated with known viruses, and underscore the importance of understanding the pathophysiology of these infections.

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Trends in Infectious Diseases: A Retrospective 5-Year Study

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Abstract

Background: Although hospital admission is frequently due to the exacerbation of chronic diseases, most often it is caused by an underlying infectious process. Patients often have several admissions per year, making them at risk for recurrent infections, increased morbidity, and the emergence of resistant strains of microorganisms.

Methods: This is a retrospective, descriptive study of all patients with an infectious disease diagnosis, who were admitted to the medical ward of a tertiary hospital during a 5-year period. Information was collected from electronic medical files regarding gender, age, autonomy, comorbidities, primary diagnosis, in-hospital length of stay, and mortality as well as microbiological data surveillance.

Results: A total of 355 patients fulfilled the inclusion criteria. Amongst the sample analysed, the average age was 78.10±12.47 years. Of the patients, 57.2% (203) were female, with most patients considered as dependent according to Katz score. The average Charlson Comorbidity Index (CCI) score was 6.28±2.74, increasing with age. The main diagnostic categories were respiratory (191 patients: 137 with pneumonia and 49 with acute bronchitis) and urinary tract (138 patients: 69 with pyelonephritis and 66 with cystitis). Urinary tract infections were more frequent in females and in dependent patients. Only 37.8% of infections had a microbiologic isolate: *Escherichia coli* (28.4%), *Pseudomonas aeruginosa* (12.7%), and *Klebsiella pneumoniae* (8.2%). The overall mortality was 6.77%.

Conclusions: The frequent in-hospital admission due to infectious diseases makes it imperative to characterise and follow-up on evolution of the disease itself in order to better know the characteristics of community-acquired diseases, establish routes of transmission and outbreak identification, microbiology patterns, and resistance towards further improving empiric therapy.

INTRODUCTION

A wide variety of infections contribute to acute care admissions to general internal medical units. There is an increasing trend in the burden of infectious disease (ID) hospitalisations, as reflected by an increase in the proportion of hospitalisations attributable to IDs.[1] IDs cause widespread morbidity and mortality worldwide. Extensive improvements in sanitation, vaccine development, and other public health measures led to a decrease in the IDs burden. However, IDs hospitalisation rates did not follow the same decrease trend in the rate of all-cause hospitalisations in the last part of the 20th century.[1-3]

The ongoing changes in the demographics due to an ageing population also pose a challenge to the departments of acute care such as internal medicine. As patients become older, the functional impairment rate and the prevalence of chronic disease increases and, as such, comorbidities (defined by the number of chronic diseases in addition to the index disease)[3] and also the susceptibility to IDs increases.

Acute care is defined by the World Health Organization (WHO) as "a range of clinical healthcare functions, including emergency medicine, and others as urgent care, and short-term inpatient stabilisation,"[4] and comprises the initial assessment, diagnosis, treatment, and discharge or transfer to an appropriate setting of patients.[2,5] Worldwide, the absolute number of acute admissions has increased, as have the acute admissions due to IDs, and this increase associated with a higher prevalence of older patients with different characteristics has challenged the structure of acute hospital care.[6]

The epidemiology of IDs is a composite of several environmental, behavioural, social, and biological variables, including the selective effect of drugs or vaccines, and other unpredictable events that can change over time and have potentially important effects on global health. Throughout the last century, there has been substantial variation in the number of hospitalisations of this disease group, namely respiratory infections, diarrhoeal diseases, HIV or AIDS, tuberculosis, and malaria, although major differences in their ranking exist between high- and low-income countries.[7,8]

Multiple studies over the past two decades have shown that morbidity and mortality due to IDs are associated with significant health care burdens and expenditures.[3,7,9] Apart from having chronic diseases, patients in the departments of internal medicine comprise a complex population, due to the varying degrees of disease severity and functional impairment.[9,10]

The overall aim is to evaluate the population admitted due to IDs in terms of patient characteristics, admission rates, main reasons for admission, and prognosis after a short-term follow-up, establishing a trend in the admissions due to infectious causes in a tertiary care centre.

METHODS

The authors performed a retrospective study on all patients with an infectious diagnosis who were admitted to an acute ward of a tertiary hospital from 2012 to 2016. Inclusion criteria were an age over 18 years and an International Classification of Diseases, Ninth Revision (ICD-9) code for infectious and parasitic diseases (001–139; 320–326; 420–429, 460–519; 590; 595; 601; 680–686; 730; 995.9; and 996–999).

The authors performed an electronic medical review and collected demographic data, patients' autonomy assessed by Katz score, comorbidities (by the Charlson Comorbidity Index [CCI]), and primary diagnosis (according to a diagnostic category). They also collected data related to microbiology and antibiotic treatment.

The authors' outcomes were length of stay, microbiological positivity, antibiotic use and switch, and mortality.

Data were analysed as non-normal, with median and interquartile range. Trends throughout the years were evaluated by linear regression for continuous normal outcomes, tests for trends for continuous non-normal variables, and standard correlation for the binary outcomes. A p-value of <0.05 was considered to be significant. Analysis was conducted with Stata 14 (StataCorp, College Station, Texas, USA).

RESULTS

From January 2012 to December 2016, the authors included a total of 2,615 patients admitted to an acute ward with an infectious diagnosis. An increase following a decrease was observed over the years (p<0.0001). The median age was overall unchanged (80 years [interquartile range: 71–87 years]; p=0.5000). However, the male prevalence decreased from 53% to 43% (p<0.0001), and a peak was observed for the CCI (p=0.0010) in 2015 (Table 1).

The major diagnostic criteria were respiratory (34.0%), genitourinary (34.0%), gastrointestinal (4.0%), soft tissue (4.0%), systemic (3.0%), bone and joint (0.7%), and central nervous system (0.3%). There was a trend towards a decrease in respiratory infections, along with an increase in genitourinary infections (p<0.0001 [Table 1]). The most frequent diagnoses were respiratory and genitourinary and followed the same trend (p=0.0080): pneumonia (38%), cystitis (18%), pyelonephritis (16%), and bronchitis (15%) (Table 1).

Table 1: Demographic data and diagnosis characterisation trends from 2012 to 2016.

	2012	2013	2014	2015	2016	Total	p
Demographic data							
Age (years)	80 (72–87)	81 (71–87)	80 (69–86)	79 (69–86)	81 (71–87)	80 (71–87)	0.5000
Gender (male)	282 (53%)	270 (53%)	231 (51%)	214 (53%)	304 (43%)	1,301 (50%)	<0.0001
CCI	6 (5–8)	6 (4–7)	6 (4–8)	7 (5–9)	6 (5–8)	6 (5–8)	0.0010
Total	532	512	457	404	710	2,615	<0.0001
Diagnostic category							
Respiratory	325 (61.0%)	257 (50.0%)	248 (54.0%)	202 (50.0%)	380 (54.0%)	1,412 (54.0%)	<0.0001
Genitourinary	139 (26.0%)	197 (39.0%)	139 (30.0%)	136 (34.0%)	278 (39.0%)	889 (34.0%)	
Gastrointestinal	28 (5.0%)	23 (4.5%)	16 (4.0%)	15 (4.0%)	20 (3.0%)	102 (4.0%)	
Soft tissue	21 (4.0%)	16 (3.0%)	20 (4.0%)	21 (5.0%)	24 (3.0%)	102 (4.0%)	
Systemic	13 (2.0%)	15 (3.0%)	27 (6.0%)	26 (6.0%)	4 (0.6%)	85 (3.0%)	
Central nervous system	1 (0.2%)	2 (0.4%)	3 (0.7%)	1 (0.3%)	0 (0.0%)	7 (0.3%)	
Diagnosis							
Pneumonia	214 (40.0%)	202 (40.0%)	166 (36.0%)	143 (35.0%)	274 (39.0%)	999 (38.0%)	0.0080
Cystitis	83 (16.0%)	100 (19.0%)	84 (18.0%)	64 (16.0%)	132 (19.0%)	463 (18.0%)	
Pyelonephritis	56 (11.0%)	94 (18.0%)	55 (12.0%)	71 (18.0%)	140 (19.0%)	416 (16.0%)	
Bronchitis	111 (21.0%)	55 (11.0%)	82 (18.0%)	57 (14.0%)	96 (14.0%)	401 (15.0%)	
Colitis/enteritis	28 (5.0%)	23 (4.0%)	16 (4.0%)	14 (3.0%)	20 (3.0%)	101 (4.0%)	
Abscess	2 (0.4%)	7 (1.4%)	9 (2.0%)	8 (2.0%)	20 (3.0%)	46 (2.0%)	
Bacteriemia/ sepsis	2 (0.4%)	4 (0.8%)	20 (4.0%)	14 (4.0%)	4 (0.6%)	44 (2.0%)	
Erysipelas	6 (1.0%)	4 (0.8%)	7 (1.5%)	10 (2.0%)	4 (0.6%)	31 (1.0%)	
Cellulitis	13 (2.0%)	5 (1.0%)	4 (1.0%)	3 (0.7%)	0 (0.0%)	25 (1.0%)	
Prostatitis	0 (0.0%)	3 (0.6%)	0 (0.0%)	1 (0.3%)	6 (0.9%)	10 (0.4%)	
Endocarditis	1 (0.2%)	2 (0.4%)	2 (0.4%)	3 (0.7%)	0 (0.0%)	8 (0.3%)	
Candidiasis	1 (0.2%)	2 (0.4%)	1 (0.2%)	3 (0.7%)	0 (0.0%)	7 (0.3%)	
Central catheter infection	3 (0.6%)	2 (0.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (0.2%)	

CCI: Charlson Comorbidity Index.

There were 45 agents identified in total, including bacteria, virus, parasites, and fungus. The top 18, with an overall prevalence over 1%, were *Escherichia coli* (29.0%), *Pseudomonas aeruginosa* (9.0%), *Klebsiella pneumoniae* (7.0%), *E. coli* extended-spectrum β-lactamase (ESBL; 6.8%), methicillin-resistant *Staphylococcus aureus* (MRSA; 6.7%), *Enterococcus* spp. (5.0%), *Proteus mirabilis* (4.2%), pneumococcus (4.1%), methicillin-sensitive *S. aureus* (3.9%), Enterobacteriaceae spp. (3.6%), *Candida albicans* (2.3%), *K. pneumoniae* ESBL (2.1%), *Clostridium difficile* (1.7%), *Candida* spp. (1.4%), *Morganella morganii* (1.3%), *Streptococcus* spp. (1.3%), influenza virus (1.2%), and *Acinetobacter baumannii* (1.1%). Of these agents, a marginally significant trend was observed (p=0.057), with a reduction in *E. coli*, MRSA, *Enterococcus* spp., and *A. baumannii*, and an increase in *P. aeruginosa*, *E. coli* ESBL, *Enterobacteriacea* spp., and *K. pneumoniae* ESBL (Table 2).

Over 30 different antibiotics were used, either alone or in combination as a first-line therapy for these infections. The 10 most frequent (overall prevalence of over 1%) were ceftriaxone (36.0%), amoxicillin/clavulanic acid (13.6%), azithromycin (13.0%), clarithromycin (11.5%), piperacillin/tazobactam (9.8%), meropenem (4.0%), levofloxacin (2.3%), ciprofloxacin (1.8%), vancomycin (1.5%), and flucloxacillin (1.0%). There was a significant trend (p=0.001) with increased use of azithromycin with a simultaneous decline in clarithromycin use. Moreover, there was also an overall rise in ceftriaxone and amoxicillin/clavulanic acid, together with piperacillin/tazobactam use, compared with a decreased use of meropenem, levofloxacin, ciprofloxacin, vancomycin, and flucloxacillin (Table 3).

Considering the outcomes (Table 3), the length of stay was, overall, unchanged (median: 8 days [interquartile range: 6–12 days]; p=0.6). Though not statistically significant, there was a growth in antibiotic switch from 16% to 23% (p=0.06). Antibiotic usage also increased from 95% to 97% (p=0.04), along with microbiologic positivity (23% to 38%; p<0.0001). Contrasting, mortality declined from 12% to 7% (p=0.001).

DISCUSSION

In the authors' short-term (5 year) assessment of IDs trends, they found that its incidence as the sole admission motive is diminishing. However, the authors have not taken into account IDs as a second diagnosis, decompensating chronic pathologies such as heart failure or chronic obstructive pulmonary disease, which could lead to underestimation despite a slight increase in the CCI, with observed a sustained population age of 80 years. Respiratory and genitourinary infections are the most prevalent, and follow an opposite tendency, with one decreasing and the other increasing. As genitourinary infections were more prevalent in elderly females with more comorbidities and with decreased functional impairment rate, that increased during the 5 years.

The most frequent agents remain bacteria, with an emphasis on Enterobacteriaceae; however, attention should be paid to the influenza virus and *Candida*, as well. It was interesting to observe the raise in ESBL Enterobacteriaceae, with concomitant decline in MRSA and *A. baumannii*. This data is in line with international data regarding these agents.[11]

As far as antibiotic use goes, there was a significant increase, which could lead to healthcare-associated infections;[12] however, the core issue remains: the use of narrow-spectrum antibiotics as first-line empiric drugs, despite the rise in frequency of in-hospital ESBL agents, which can be related to the authors' infection control programme efforts. The shift from clarithromycin to azithromycin was the result of changes of the hospital protocol for respiratory infections, from the infection control programme. This in-hospital programme elaborates a series of acting protocols regarding suggested antibiotics for common infections, together with annual reports regarding microbiology resistances. They also implement infection control measures such as hand-washing inspections, aseptic measures, and control over broad-spectrum antibiotics.

One of the most striking outcome results was the increase in microbiologic positivity from 23% to 38%, justified by adequate collection of cultural samples and the continuous improvement of laboratory techniques, which helps to proceed with antibiotic switch or maintenance more accurately. Also of note is the decrease of in-hospital mortality, without significative shift in the hospital length of stay, which could be related to both a higher and accurate use

Table 2: Microbiologic isolation and antibiotic use trends from 2012 to 2016.

	2012	2013	2014	2015	2016	Total	p
Microbiology							
E. coli	42 (34.0%)	56 (30.6%)	63 (35.2%)	45 (32.0%)	76 (28.4%)	282 (29.0%)	0.057
Pseudomonas aeruginosa	11 (8.9%)	15 (8.2%)	15 (8.4%)	10 (7.1%)	34 (12.7%)	87 (9.0%)	
K. pneumoniae	10 (8.1%)	10 (5.5%)	12 (6.7%)	14 (9.9%)	22 (8.2%)	68 (7.0%)	
E. coli ESBL	1 (0.8%)	15 (8.2%)	23 (12.8%)	9 (6.4%)	18 (6.7%)	66 (6.8%)	
MRSA	9 (7.3%)	18 (9.8%)	11 (6.1%)	9 (6.4%)	18 (6.7%)	65 (6.7%)	
Enterococcus spp.	8 (6.5%)	16 (8.7%)	6 (3.4%)	5 (3.5%)	14 (5.2%)	49 (5.1%)	
Proteus mirabilis	8 (6.5%)	12 (6.6%)	2 (1.1%)	3 (2.1%)	16 (6.0%)	41 (4.2%)	
Pneumococcus	5 (4.1%)	8 (4.4%)	7 (3.9%)	10 (7.1%)	10 (3.7%)	40 (4.1%)	
MSSA	5 (4.1%)	5 (27%)	8 (4.5%)	8 (5.7%)	12 (4.5%)	38 (3.9%)	
Enterobacteriaceae spp.	2 (1.6%)	3 (1.6%)	6 (3.4%)	8 (5.7%)	16 (6.0%)	35 (3.6%)	
Candida albicans	1 (0.8%)	4 (2.2%)	0 (0.0%)	0 (0.0%)	12 (4.5%)	23 (2.4%)	
K. pneumoniae ESBL	0 (0.0%)	4 (2.2%)	1 (0.6%)	2 (1.4%)	14 (5.2%)	21 (2.2%)	
Clostridium difficile	3 (2.4%)	4 (2.2%)	0 (0.0%)	2 (1.4%)	2 (0.7%)	17 (1.8%)	
Candida spp.	2 (1.6%)	12 (6.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	14 (1.4%)	
Morganella morganii	1 (0.8%)	4 (2.2%)	2 (1.1%)	0 (0.0%)	6 (2.2%)	13 (1.3%)	
Streptococcus spp.	2 (1.6%)	1 (0.5%)	6 (3.4%)	4 (2.8%)	0 (0.0%)	13 (1.3%)	
Influenza virus	0 (0.0%)	1 (0.5%)	0 (0.0%)	1 (0.7%)	10 (3.7%)	12 (1.2%)	
Acinetobacter baumannii	7 (5.7%)	2 (1.1%)	1 (0.6%)	1 (0.7%)	0 (0.0%)	11 (1.1%)	
Antibiotics							
Ceftriaxone	268 (37.4%)	243 (37.7%)	224 (37.5%)	186 (38.0%)	338 (39.0%)	1,259 (36.0%)	0.001
Amoxicillin/ clavulanic acid	73 (10.2%)	81 (12.6%)	91 (15.2%)	77 (15.7%)	154 (17.8%)	476 (13.6%)	
Azithromycin	9 (1.3%)	23 (3.6%)	107 (17.9%)	113 (23.1%)	202 (23.3%)	454 (13.0%)	
Clarithromycin	206 (28.8%)	138 (21.4%)	43 (7.2%)	3 (0.6%)	10 (1.2%)	400 (11.5%)	
Piperacillin/ tazobactam	57 (8.0%)	60 (9.3%)	50 (8.4%)	68 (13.9%)	108 (12.5%)	343 (9.8%)	
Meropenem	34 (4.7%)	45 (7.0%)	33 (5.5%)	14 (2.9%)	22 (2.5%)	148 (4.2%)	
Levofloxacin	24 (3.4%)	16 (2.5%)	18 (3.0%)	11 (2.2%)	12 (1.4%)	81 (2.3%)	
Ciprofloxacin	24 (3.4%)	18 (2.8%)	11 (1.8%)	2 (0.4%)	8 (0.9%)	63 (1.8%)	
Vancomycin	12 (1.7%)	14 (2.2%)	12 (2.0%)	5 (1.0%)	8 (0.9%)	51 (1.5%)	
Flucloxacillin	9 (1.3%)	7 (1.1%)	8 (1.3%)	10 (2.0%)	4 (0.5%)	38 (1.1%)	

ESBL: extended-spectrum β-lactamase; *E. coli*: *Escherichia coli*; *K. pneumoniae*: *Klebsiella pneumoniae*; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*.

Table 3: Outcome trends from 2012 to 2016.

Outcomes	2012	2013	2014	2015	2016	Total	p
Length of stay (days)	7 (5.0–12.0)	8 (4.0–12.5)	8 (5.0–12.0)	8 (6.0–12.0)	7 (6.0–12.0)	8 (6.0–12.0)	0.6000
Microbiological positivity	123 (23%)	183 (35%)	179 (39%)	141 (15%)	268 (38%)	894 (34%)	<0.0001
Antibiotic use	508 (95%)	487 (95%)	443 (97%)	391 (97%)	690 (97%)	2,519 (96%)	0.0400
Antibiotic switch	65 (16%)	124 (24%)	78 (17%)	73 (19%)	164 (23%)	524 (20%)	0.0600
Mortality	63 (12%)	57 (11%)	60 (13%)	35 (9%)	50 (7%)	265 (10%)	0.0010

in antibiotics, the increase in microbiologic positivity, and infection control policies. The author's decrease in mortality contrasts with data from South Korea, which documented an increase in mortality in the elderly population.[13]

The authors' main bias is the short duration of the study, as 5 years can be insufficient for some trends to establish as well as its retrospective nature. Also, it would have been of interest to perform some economic analysis on the burden of these tendencies as to help in health policies changes. However, the authors do believe this analysis is important, even if just on a local point of view, to establish prevalences and resistances.

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Aspergillus terreus Peritonitis in a Child on Continuous Ambulatory Peritoneal Dialysis: A Case Report from Pakistan

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Abstract

Background: *Aspergillus* peritonitis is a rare, potentially fatal complication of continuous ambulatory peritoneal dialysis (CAPD). Several cases of fungal peritonitis (FP) caused by *Aspergillus* species have been reported worldwide; however, there is a paucity of data from Pakistan. Here, a case of congenital nephrotic syndrome is reported in a patient who progressed to Stage 5 chronic kidney disease when she was 8 years old, which was managed by CAPD and other supportive therapies. The patient developed FP and later succumbed to death despite appropriate management. Early diagnosis and careful interpretation of culture results are, therefore, important for the treatment of these patients.

Case presentation: The patient outlined in this case report had congenital nephrotic syndrome, Stage 5 chronic kidney disease, and was on CAPD. She presented with peritonitis. Initially, broad spectrum antibiotics were started, and peritoneal samples did not yield any growth. Later, consecutive peritoneal samples taken from the patient grew *Aspergillus terreus*, with septate hyphae seen on a direct smear from the sample. Voriconazole was started immediately, and the patients' catheter was removed. However, the patient's condition deteriorated and, after prolonged intensive care and high ventilator support, the patient expired.

Conclusion: *A. terreus* is an uncommon and deadly pathogen that does not respond to the commonly used antifungal treatments such as amphotericin B. The treatment of CAPD-related FP requires early diagnosis and the use of newer drugs such as voriconazole or caspofungin.

BACKGROUND

Continuous ambulatory peritoneal dialysis (CAPD) is a well-known management technique for paediatric Stage 5 chronic kidney disease (CKD-5) used worldwide.[1,2] However, the majority of children in Pakistan are dialysed by haemodialysis (HD), and very few receive successful kidney transplants. CAPD is rarely practiced in Pakistan due to the scarcity of costly dialysis consumables and a lack of education and trained staff.[3,4] Peritonitis and catheter-related infections are a major cause of CAPD failure, resulting in switching to HD or death. Although bacterial peritonitis (BP) is the most common complication, fungal peritonitis (FP) is a rare and serious complication of CAPD. The risk factors for FP include recurrent BP, use of broad-spectrum antibiotics, prolonged hospitalisation, underlying proteinuria, immunosuppression, prolonged duration of CAPD, and frequent manipulation of the peritoneal dialysis (PD) catheter. FP is an indication of switching to HD and removing the catheter.

Several cases of FP caused by *Aspergillus* species (spp.) have been reported worldwide; however, there is a paucity of data from Pakistan.[5-8] Here, a case of congenital nephrotic syndrome (CNS) is reported in a patient who progressed to CKD-5 when she was 8 years old, which was managed by CAPD and other supportive therapies. She developed recurrent episodes of BP and ultimately FP and succumbed to death despite use of aggressive systemic and intraperitoneal (IP) antibiotics and antifungal agents, removal of the PD catheter, switching to HD, and receiving intensive ventilator care. Early diagnosis and careful interpretation of culture results are therefore important for the treatment of these patients.

CASE PRESENTATION

The patient in this case report was an 8-year-old female who had CNS since she was 1 month old. Her younger brother also had CNS and had previously expired. She was diagnosed with CNS at another hospital, where she was managed with intravenous albumin, along with diuretics administered periodically. However, genetic or immunological investigations or a kidney biopsy were not carried out. The patient was treated with enalapril and oral diuretics, but not with steroids or any other immunosuppressant. She was asthmatic since early childhood, requiring frequent nebulisation, and had been on and off on montelukast. The patient underwent conservative treatment for CKD at the primary institute for almost 15 months but, due to persistent symptomatic asthma not fulfilling the general fitness required for anaesthesia, long-term vascular access could not be constructed, and she reached CKD-5. Despite use of thyroxine supplements, she exhibited developmental delay with hyperactive behaviour.

The patient was referred to the authors' institute for CAPD in September 2020. The clinical details of the patient are listed here (Table 1). The patient was diagnosed with generalised oedema, hypoalbuminaemia, severe hypertension, severe anaemia, and symptomatic uraemia, with a serum creatinine level of 11.68 mg/dL and an estimated glomerular filtration rate of 5.26 mL/min/1.73 m^2, confirming CKD-5. Her growth parameters (weight: 18 kg; height: 112 cm) were below the third centile for her age group, suggesting long-standing CKD. The patient underwent management of hypertension, anaemia, and uraemia with acute HD. After the laparoscopic placement of a Tenckhoff catheter, CAPD was initiated; the CAPD prescription was 5 exchanges/day with a fill volume of 400 mL of 1.5% dextrose and ultrafiltration of 350–400 mL.

The patient had recurrent episodes of BP during CAPD, as suggested by the high cell count in the PD fluid analysis, though the cultures remained negative (Table 1). Each episode was treated with IP antimicrobials, with clinical and PD fluid count improvement. Lastly, she presented with high-grade fever with associated chills and rigors for 5 days. Initially, IP antibiotics were started after sending PD-effluent samples for analysis and culture, but the patient's condition did not improve after 7–10 days. She remained sick, with continuous dull pain, fever, and occasional vomiting, and her levels of C-reactive protein increased from 24 to 48 mg/L. PD fluid analysis showed a very high cell count (1,225 /mm^3) with more than 90% polymorphs. Bacterial cultures and the acid-fast bacilli smear were negative.

Table 1: The characteristics of the patient outlined in this case report with *Aspergillus terreus* peritonitis on continuous ambulatory peritoneal dialysis for Stage 5 chronic kidney disease.

Age: 8 years Gender: Female Weight (kg): 18 Height (cm): 112 Body surface area (m^2): 0.74	Age at CNS diagnosis: 1 month First visit to centre: 24th September 2020 Vitals: Respiratory rate (/min): 20 Blood pressure (mmHg): 144/92 Body temperature (°C) : 35.8			Diagnosis: CKD-5 Cause: CNS Dialysis initiation: 24th September 2020		
Month/Year	September–October 2020	November 2020	December 2020	January 2021	February 2021	March–April 2021
Dialysis modality	HD	CAPD				HD
Access type	Femoral	Peritoneal catheter				Internal jugular
Access placement and removal	Femoral line: 24th September 2020	PD catheter placement: 1st October 2020				Temporary: 2nd March 2021
		PD catheter removal: 3rd March 2021				Permcath: 16th March 2021
PDF: detailed report						
Protein (g/dL)	0.25	0.31	0.20	0.29	0.04	0.20
LDH (U/L)	N/A	N/A	N/A	155	74	95
Glucose (mg/dL)	1,035	376	377	188	914	1,300
Gram stain	Negative	Negative	Negative	Negative	Negative	Negative
TLC (cells/cmm)	340	50	375	105	1,225	2,000
Neutrophils (%)	25	80	5	10	90	90
PDF culture	Negative	Negative	Negative	Negative	Negative	Negative
PDF acid-fast bacillus smear					Negative	
PDF fungal smear				Negative	Positive	
PD fungal culture				Negative	*A. terreus*	
Blood culture	Negative	Negative	Negative	Negative	Negative	Negative
Serum galactomannan						Negative

Table 1 continued.

Month/Year	September 2020	October 2020	November 2020	December 2020	February 2021	March–April 2021
Haemoglobin (g/dL)	6.5	12.0	13.7	9.8	10.6	9.1
TLC (cmm)	14,200	14,800	5,150	5,940	15,000	14,950
Neutrophils (%)	62	72	65	60	66	68
Platelets (cmm)	359	408	308	157	474	317
C-reactive protein	High	High	High	High	High	High
Urea (mg/dL)	185	97	122	14	127	107
Creatinine (mg/dL)	5.26	6.60	7.14	7.80	7.80	5.40
eGFR (mL/min/1.73 m^2)	138.00	9.33	8.62	7.89	7.89	11.40
Serum sodium (mEq/L)	4.5	136.0	140.0	139.0	144.0	141.0
Serum potassium (mEq/L)	14.0	3.5	4.5	4.7	3.5	3.8
Serum bicarbonate (mEq/L)	2.9	22.0	21.0	22.0	23.0	19.0
Albumin (g/dL)	6.91	3.49	2.09	1.90	2.80	N/A
Calcium (mg/dL)	7.57	8.60	8.90	7.30	8.10	8.28
Phosphorus (mg/dL)	108.00	4.64	3.41	6.40	9.70	5.6
Alkaline phosphatase (U/L)	N/A	N/A	N/A	N/A	170	N/A
Parathyroid: (pg/mL)	269					
Spot urine protein/creatinine ratio: (mg/mg)	32.80		23.71			
Kidney ultrasound	Bilateral small kidneys (right kidney: 5.7x2.3 cm; left kidney: 4.8x2.1 cm). Increased echogenicity with loss of corticomedullary distinction					
Echocardiography	Moderately dilated left atrium; preserved ventricular function; and mild pulmonary hypertension. Ejection fraction: 50%					
COVID-19 PCR	Negative					Negative

A. terreus: *Aspergillus terreus*; CAPD: continuous ambulatory peritoneal dialysis; CKD-5: Stage 5 chronic kidney disease; CNS: congenital nephrotic syndrome; eGFR: estimated glomerular filtration rate; HD: haemodialysis; LDH: lactate dehydrogenase; N/A: not applicable; PD: peritoneal dialysis; PDF: peritoneal dialysis fluid; TLC: total leukocyte count.

Subsequent dialysate effluent samples revealed septate hyphae on microscopy with 3% potassium hydroxide. After 3–5 days of incubation at 37 °C, Sabouraud dextrose agar yielded buff tan, cinnamon brown, velvety, powdery colonies with a yellow tan reverse (Figure 1). Microscopic examination with lactophenol cotton blue solution revealed septate hyphae; smooth, relatively short conidiophores; and vesicles with smooth, round conidia on compactly columnar, biseriate phialides, equal in length to the metulae and covering the upper half of the vesicle. It was identified phenotypically as *Aspergillus terreus* (Figure 2).[9]

Figure 1: Macroscopic colony morphology of *Aspergillus terreus* culture on Sabouraud dextrose agar.

The colonies observed were buff tan, cinnamon brown, velvety, and powdery, with a yellow tan reverse.

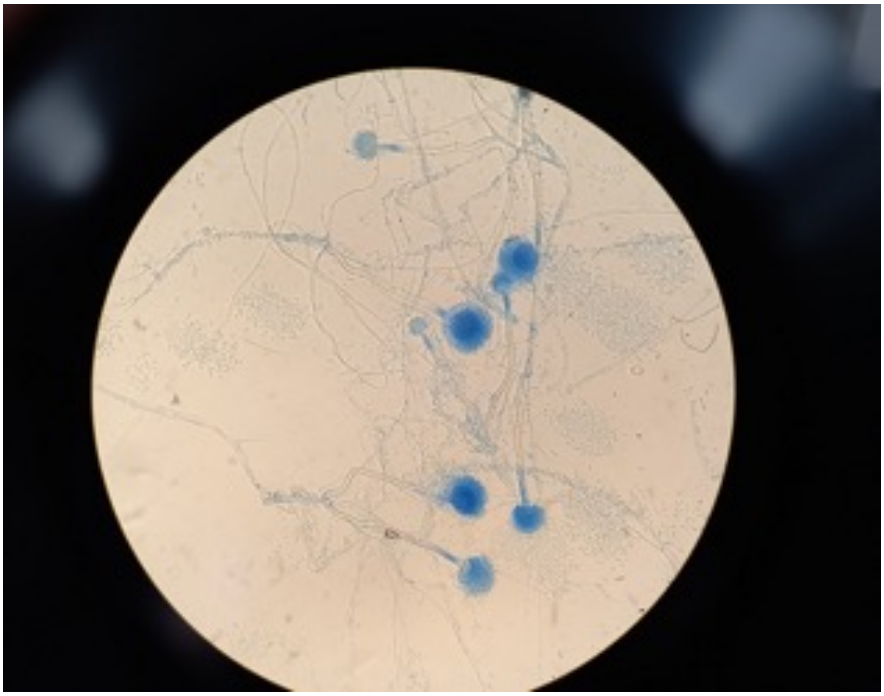

Figure 2: Microscopic appearance of *Aspergillus terreus*, stained using lactophenol cotton blue solution, on Sabouraud dextrose agar (magnification x40).

This image shows compactly columnar, biseriate phialides covering the upper half of the vesicle.

Voriconazole was started and continued in the recommended systemic and intraperitoneal dose, the CAPD catheter was removed, and the patient was switched to HD through a temporary double-lumen catheter, which was later replaced by a permcath. However, after 2–3 weeks, she presented with hypertensive encephalopathy and respiratory distress. Her serum galactomannan test was negative, so invasive aspergillosis was ruled out. The patient's condition deteriorated, requiring mechanical ventilator support, and her Glasgow Coma Scale (GCS) score declined. After a prolonged stay under intensive care and high ventilator support, the patient developed ventilator-associated pneumothorax and subsequently expired.

DISCUSSION

The authors describe a case of FP with *A. terreus* in an 8-year-old child from Pakistan with CKD-5 secondary to CNS on CAPD. From the authors' experience, this is the first reported case of a CNS patient who survived and underwent CAPD, since most children die during the first 2–3 years of their diagnoses, often as a result of intercurrent infections rather than uraemia. Though CNS was not confirmed genotypically but was onset during early infancy, due to the death of the patient's younger brother from CNS and the persistence of oedema, nephrotic range proteinuria, and hypoalbuminaemia in the patient, her progression to CKD-5 at this age strongly favours the diagnosis of CNS.

FP accounts for 3–10% of cases of CAPD-related peritonitis.[6,10,11] In a study from Saudi Arabia, patients with CNS and those younger than 5 years old had a higher incidence of FP, with a higher rate of catheter removal than in patients diagnosed with BP.[12] FP is mostly caused by *Candida* spp. In a study from India, among 142 episodes of peritonitis, 20 (14%) cases were due to FP.[13] Prior exposure to antibiotics was seen in nine cases, 18 cases were due to *Candida* spp., 12 cases recovered and CAPD was re-initiated, the modality was changed to HD in four cases, and the patients expired in four cases.[13] Filamentous fungi less frequently cause FP; however, different types of filamentous fungi have been reported to cause peritonitis, including *Aspergillus*, *Paecilomyces, Penicillium,* and *Acremonium* spp.[5,7,14] Although *Aspergillus* spp. rarely cause FP (2–5% of cases), it has a high morbidity and mortality rate and its diagnosis and treatment is challenging.[5,8,14] Multiple *Aspergillus* spp. have been reported in FP, including *A. terreus, Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Aspergillus sydowii,* and *Aspergillus oryzae*.[6,14] *Aspergillus* spp. is an environmental organism and may enter the peritoneal cavity via dialysis catheters.

Factors like malnutrition, hypoalbuminaemia, left ventricular dysfunction, and anaemia are well-established risk factors for high mortality in children on dialysis.[5] Persistent nephrotic range proteinuria and hypoalbuminaemia, poor dietary intake, asthma, CKD-5 requiring dialysis, malnutrition, recurrent hospitalisation, and systemic and IP antibiotic use for recurrent peritonitis and invasive procedures, may have contributed to the patient's fatality. Prolonged duration of PD (more than 36 months) may be associated with *Aspergillus* peritonitis.[15] Although the patient received PD for only a few months, a delayed diagnosis and the persistence of catheter infection may have led to death in this case.

Several cases of FP caused by *Aspergillus* spp. have been reported worldwide; however, there is a paucity of data from Pakistan.[5-8] Data from a study of 42 patients on acute and chronic PD showed 35 episodes of peritonitis (83.34%), with 11.00% diagnosed as FP and one of these FP cases due to *Aspergillus* spp.[16] Due to limitations of diagnostic modalities, the authors' diagnosis of CNS and FP could not be confirmed by molecular methods.

CONCLUSION

FP due to *Aspergillus* spp. is associated with high morbidity and mortality. *A. terreus* is an uncommon and deadly pathogen that does not respond to the common antifungals, including amphotericin B. Despite its difficult diagnosis and rare occurrence, an early diagnosis by culture using multiple samples of PD effluent, as was the case in the authors' setup, may help in the decision to remove the PD catheter and to use newer drugs such as voriconazole or caspofungin. Early diagnosis and careful interpretation of culture results are, therefore, important for the treatment of these patients.

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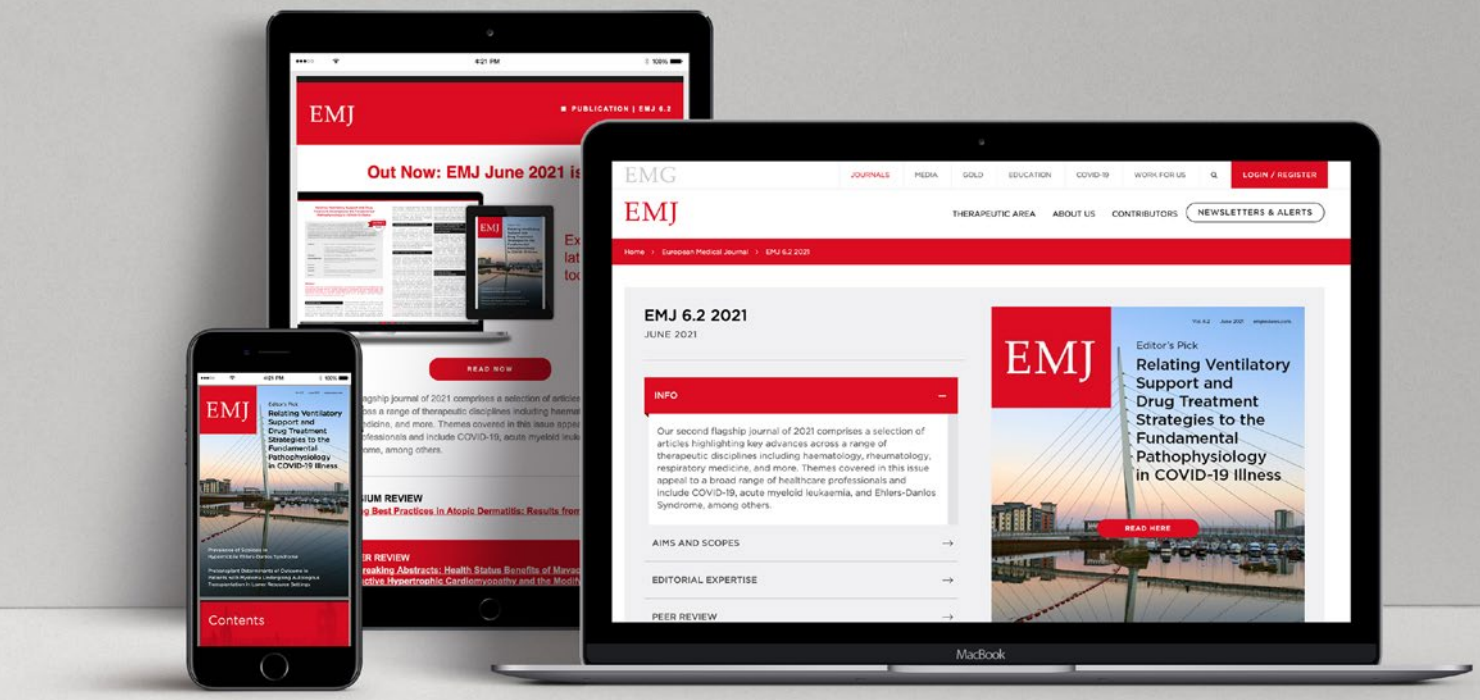

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