EMJ

Editor's Pick

Relating Ventilatory Support and Drug Treatment Strategies to the Fundamental Pathophysiology in COVID-19 Illness

Prevalence of Scoliosis in Hypermobile Ehlers-Danlos Syndrome

Pretransplant Determinants of Outcome in Patients with Myeloma Undergoing Autologous Transplantation in Lower Resource Settings



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January 2021 MAT-40487

1. Lebwohl M, et al. N Engl J Med 2015;373:1318–28. 2. Kyntheum® (brodalumab) EU Summary of Product Characteristics. July 2020. Available from: https://www.ema.europa.eu/en/medicines/human EPAR/kyntheum. Last Accessed: January 2021. 3. Brembilla NC et al. Front Immunol 2018;9:1682. 4. Pappu R et al. Immunology 2011;134:8–16. 5. Baker KF and Isaacs JD. Ann Rheum Dis 2018;77:175–87.







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Spencer Gore, CEO

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VIEW IN FULL \leftarrow

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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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Welcome

Dear Readers,

It is my great pleasure to introduce the newest issue of our multidisciplinary flagship journal, *EMJ*, which brings together the latest advancements across the medical field that matter most. These pages contain a promising selection of peer-reviewed studies and review articles covering therapeutic areas such as cardiology, dermatology, respiratory medicine, and rheumatology.

As coronavirus disease (COVID-19) continues to spread around the world, we are publishing a topical article by Lewis et al., which relates ventilatory support and drug treatment strategies to the fundamental pathophysiology in COVID-19 illness. The authors explain why understanding the mechanisms and timings of alveolar damage can allow for tailored interventions. As well as being highly relevant, this comprehensive review is also the Editor's Pick for our latest issue of *EMJ*.

For the haematologists amongst you, we have the review by Nair on the pretransplant variables that impact the outcome of autologous stem cell transplantation in patients with multiple myeloma. Although the presence of high-risk cytogenetics is considered the main adverse factor predicting shorter survival, there are numerous other potential determinants associated with post-transplant outcomes that are explored in this fascinating paper. Nair also delineates the growing interest in minimal residual disease measurements as a potent prognostic marker in myeloma.

In addition, a timely piece by Russell et al. explores the rationale behind the use of oxyhydrogen gas as a novel and sustainable treatment for COVID-19. The authors outline how a combination of hydrogen and oxygen can affect cellular processes at the molecular level, focussing on the evolutionary requirement for these gases. This is coupled to an investigation of emerging preclinical and clinical data concerning the safety and efficacy of this approach in treating inflammatory-related disorders. This review has both theoretical and practical significance, which will appeal to both pulmonologists and a wider healthcare audience alike.

Finally, I would like to thank all the contributors and expert authors for their valued collaboration and input, and extend my appreciation to the entire Editorial Board for their dedication and hard work during these difficult times. I hope you, the reader, enjoy the important research inside this flagship issue of *EMJ*.



Spencer Gore Chief Executive Officer, EMG-Health



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QULAL DEL

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Foreword

Dear Colleagues,

It is my pleasure to present the latest issue of the flagship journal, *EMJ*. The overarching theme is coronavirus disease (COVID-19) infections, focussing on pathophysiologic mechanisms in order to facilitate a targeted management approach. A variety of articles on COVID-19, as well as other disease areas, are presented.

The Editor's Pick for this issue is a review paper titled: "Relating Ventilatory Support and Drug Treatment Strategies to the Fundamental Pathophysiology in COVID-19 Illness" by Lewis et al. COVID-19, which induces multiorgan disease and lung pathology, is a major cause of intensive therapy unit admissions. Pathophysiologic and radiologic features of alveolar inflammation, subsequent secondary or concurrent endothelial infection and dysfunction, and coagulopathy are reviewed. Understanding the basic disease mechanisms and timings of alveolar damage can inform the nature of ventilatory support required and the timing of targeted pharmacotherapies.

Russell et al. review the use of oxy-hydrogen gas, a gaseous mixture of molecular hydrogen and oxygen, as a treatment in COVID-19 lung inflammation. Reactive oxygen species promote inflammation, especially in hypoxic environments. Oxy-hydrogen gas reduces oxidative stress, attenuates inflammation, and is cytoprotective. It may also have therapeutic effects in the management of other lung inflammatory conditions, such as asthma and chronic obstructive pulmonary disease.

COVID-19 infection is associated with a high frequency of thromboembolic phenomena. A systematic review and meta-analysis of thromboembolic complications is presented by Pergola et al. Furthermore, Galván-Casas et al. present a fascinating case report, in which the most striking COVID-19 manifestation was dermatological.

Other disease areas covered include pretransplant determinants of outcomes following autologous stem cell transplantation multiple myeloma; rifampicin-induced in thrombocytopenia; prevalence of scoliosis hypermobile Ehlers-Danlos syndrome; in novel therapies for acute myeloid leukaemia; musculoskeletal brucellosis in the United Arab Emirates; and an audit review on emergency oxygen prescribing.

In conclusion, the boundaries of disease pathophysiology continue to expand. I thank all the authors and peer reviewers for committing time to this issue, despite the pressures of COVID-19.



Prof Ian C Chikanza

Consultant in Adult and Paediatric Rheumatology, Department of Rheumatology, Barts Arthritis Centre, Barts and the Royal London Hospital, London, UK; Professor of Medicine, Catholic University, and Professor in Rheumatology and Immunology, University of Zimbabwe, Harare, Zimbabwe

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

This virtual symposium took place on the 29th October 2020, as part of the 29th European Academy of Dermatology and Venereology (EADV) Virtual Congress

Chairperson:	Emma Guttman ¹
Speakers:	Emma Guttman, ¹ Mette Søndergaard Deleuran, ² Eric Simpson ³
	 Mount Sinai School of Medicine, New York City, New York, USA Aarhus University, Aarhus, Denmark Oregon Health & Science University, Portland, Oregon, USA
Disclosure:	Prof Guttman has received consultancy fees from AbbVie, Aditum Bio, Almirall, Amgen, Arena Pharmaceuticals, Asana, AstraZeneca, Bluefin, Boehringer Ingelheim, Boston Pharmaceuticals, Bristol Myers Squibb, Cara Therapeutics, Celgene, DBV Technologies, Dermira, EMD Serono (Merck & Co), Eli Lilly and Company, Evidera, Galderma, Ichnos Sciences, Janssen, Kyowa Kirin, LEO Pharma, LARRK Bio, Medscape, Novartis, Pfizer, Principia Biopharma, RAPT, Realm, Regeneron, SATO Pharmaceutical, Seanergy Dermatology, Seelos Therapeutics, Serpin Pharma, Siolta, Sonoma Biosciences, Vanda Pharmaceuticals, Ventyx Biosciences, Vimalan Biosciences, and Sanofi-Aventis; and has been a member of the scientific advisory board for Sanofi-Aventis. Prof Deleuran has received grants and personal fees, including research support, consulting/advisory board agreements, and/or honoraria for lectures, from AbbVie, Almirall, Eli Lilly and Company, LEO Pharma, Meda Pharma, Pfizer, Pierre Fabre, Regeneron, and Sanofi Genzyme. Prof Simpson has received grants and/or personal fees from AbbVie, Dermira, Eli Lilly and Company, Forte Bio Rx, Incyte, Kyowa Kirin, LEO Pharma, Menlo Therapeutics, Merck, Novartis, Ortho Dermatologics, Pfizer, Regeneron, and Sanofi Genzyme.
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Meeting Summary

Pioneering global best practices in atopic dermatitis (AD) and implementing them in the clinic are important steps towards optimising patient care. This satellite symposium, held as part of the 2020 European Academy of Dermatology and Venereology (EADV) Virtual Congress, featured a panel of leading dermatology experts who discussed key findings from the recently published Atopic Dermatitis Quality of Care Initiative.

Purpose and Mission of the Atopic Dermatitis Quality of Care Initiative

Professor Emma Guttman

For many years, AD has been deprioritised in favour of services deemed more medically urgent. New systemic therapies that are able to effectively treat both the disease and its comorbidities have also become available. More recently, patient advocates have taken important steps to increase awareness of AD. However, to reap the full benefits from these changes, Prof Guttman explained that AD care itself must also evolve.

Against this backdrop, the Global Atopic Dermatitis Quality of Care Initiative was conceived.¹ Its vision is to demonstrate the imperative for, and to improve the quality of, AD care worldwide. The mission of the initiative is to catalogue, analyse, and report the challenges to and best practices for quality AD care from renowned healthcare centres around the world. These key findings will then be disseminated to educate other healthcare providers on the priorities and best practices needed to improve and maintain quality care in AD.

The Global Atopic Dermatitis Quality of Care Initiative was overseen by an international steering group consisting of four leading AD experts with responsibility for guiding the initiative, providing practical clinical input, and evaluating the ensuing information. The study was conducted by KPMG, who carried out the collection, study, analysis, and interpretation of the data, as well as the preparation of the final report, which was commissioned and funded by Sanofi Genzyme and Regeneron.

The methodology of the initiative involved a five-step process.¹ Firstly, a stringent literature review was carried out to understand current challenges and good practices in AD care. This was augmented by in-person site visits to leading international centres using structured interviews to document key data with the aim of learning good practice directly from medical leaders in the field. A total of 32 centres from 17 countries around the world were visited, including sites in the USA, Europe, South America, and Asia.

The next step was the documentation of good practice interventions for AD care using specially created centre-specific reports and good practice case studies. These findings were then reviewed by the steering committee to ensure challenges and good practices were accurately captured and articulated. The final stage in the process was preparation of the finalised, comprehensive report, which is available online.

Global Challenges of Atopic Dermatitis and the Opportunities They Present to Improve Quality Care

Professor Mette Deleuran

Prof Deleuran discussed the four main challenges to quality of care that currently exist across the patient pathway in AD.

The first challenge is misconceptions regarding the causes and triggers of AD; people with AD can experience stigmatisation and isolation because of misconceptions that AD is contagious and are frequently affected by social and sexual issues. AD is also underappreciated by patients and often dismissed as simply a childhood disease. Even for healthcare professionals (HCP), AD can be difficult to explain and prone to misconceptions, not least because physicians themselves may have received limited training on the disease despite its prevalence. These issues are illustrated by findings from the Global Atopic Dermatitis Quality of Care Initiative, in which the vast majority of centres (91%) acknowledged that patient knowledge of, and education on, AD is lacking. Over one-half of centres (56%) also reported a need for dedicated programmes to further enhance HCP education.¹ Collectively. these medical and societal misconceptions around AD can often combine to exert a negative impact on a patient's quality of life (QoL).

The second key obstacle to quality of care in AD is delayed referral and access to AD specialists. Across healthcare systems, primary care referral is typically required to secure access to an AD specialist; however, the referral process itself is often inefficient and swamped by overwhelming demand. Prof Deleuran explained that in many parts of the world it can be very difficult, or even impossible, to obtain an appointment with an AD specialist. Consequently, primary care physicians (PCP) remain the HCP most likely to encounter patients with AD, and misdiagnosis commonplace. Several different diseases is can mimic AD presentation, and individual patients often display widely disparate clinical manifestations. Accurately determining AD severity can prove particularly challenging for PCP. Together, these gaps in PCP education and healthcare system capabilities may hinder the ability of patients to receive timely referral and access to AD specialists.

The third critical issue is poor patient access to AD treatments coupled with suboptimal adherence. AD is associated with a significant treatment burden and patients often grow tired of regular and rigorous application of topicals that can be greasy, malodorous, and damaging to clothing. Fear of treatment side effects, in particular corticophobia, is another issue that can drive poor adherence and underdosing. Evidence indicates that corticophobia, which relates to worries and negative beliefs concerning topical corticosteroids, is present among both parents of children with AD and the HCP involved in caring for them.² In some healthcare settings, AD treatment may also carry a direct financial burden for patients. For HCP, time constraints are a perpetual problem resulting in limited capacity for patient education, particularly on dosage expectations. Prof Deleuran conceded that the biggest challenge in a busy practice can often be finding time to talk to patients and parents and answer all of their questions. In this respect, nurses can prove a vital resource for improving channels of communication with patients. Within healthcare systems, there may be limited, variable, or indeed any coverage of or reimbursement for AD therapies, with limited treatment options paediatric patients. Collectively, for these shortcomings across healthcare systems lead to limited treatment options and access for patients with AD.

The fourth and final challenge to quality of care in AD is managing disease complexity and comorbidities to reduce the burden on patients. Prof Deleuran explained that there has been an increasing realisation in recent years that AD is a multisystem disease driven by type 2 inflammation and is associated with multiple comorbidities. It is important for HCP to recognise and respond to such potential comorbidities, including asthma and allergic rhinoconjunctivitis, as well as mental health disorders, such as anxiety, depression, and suicidal ideation. For patients with AD, comorbidities can have an impact on multiple components of their QoL. The lifelong requirement for medical management also exerts a heavy toll on patients. Within healthcare systems, managing complexity of AD and its comorbidities may be confounded by limited availability or access to relevant specialists within the setting of a multidisciplinary team (MDT). The overall result is that HCP, patients, and healthcare systems alike are all adversely impacted by the intricate nature of AD health management.

Good Practice Implementation Priorities that Optimise Quality of Care in Atopic Dermatitis

Professor Eric Simpson

The Atopic Dermatitis Quality of Care Initiative identified key challenges in achieving quality of care in AD that resonated with HCP globally. Prof Simpson reviewed five data-driven, good practice interventions devised to address these challenges and highlighted the important benefits they may yield. Interventions were organised into easy, difficult, and advanced steps that practising physicians can take to improve overall clinical care for their patients with AD.

Intervention 1 related to the clinical assessment and diagnosis of AD; easy steps that physicians could take to improve this aspect of care include taking a more nuanced clinical history, performing a thorough clinical assessment and evaluation of treatment response (what has worked, what has not, and why), and taking into consideration the psychological impact of AD. Prof Simpson stressed the importance of clinical assessment that encompasses the whole patient, focussing on accurately evaluating AD severity and understanding key disease drivers. More difficult next steps could include additional diagnostic assessments, such as patch testing for challenging cases or biopsy to rule out cutaneous T-cell lymphoma; shorter disease assessment tools; and consideration of the holistic impact

of AD. Advanced interventions may involve diagnostic criteria assessments, comprehensive disease assessment tools, and a focus on the long-term impact of AD.

Prof Simpson highlighted patient-reported outcomes as particularly useful assessment tools for clinicians and noted that validated 'AD control' instruments are now available online that can help to objectively determine if a patient's disease is well controlled. The potential benefits of improved clinical assessment and diagnosis for patients with AD include more timely and accurate diagnosis, guicker access to care, faster symptom relief, and improved QoL. In turn, HCP and healthcare systems stand to benefit from a reduced burden of misdiagnosis and costs, alongside an optimised disease management approach. Prof Simpson concluded that, above all, it is vital to ensure patients with AD receive a timely and accurate evaluation, diagnosis, and assessment of their disease using established instruments which will, in turn, accelerate access to care and prevent disease progression.

Intervention 2 aimed to wield the advantages afforded by a co-ordinated and structured MDT. Easy steps to enhance MDT collaboration include identifying appropriate specialists, pinpointing those providers who would make good AD team members, and assessing relevant comorbidities in clinical consultations. More difficult MDT implementation strategies could involve participating in meetings and training with external specialists, and collaborating in cross-speciality research. Multidisciplinary clinics, cross-speciality units, specialist patient teleconsultations, and cross-speciality patient group events represent examples of advanced MDT collaboration. Prof Simpson highlighted a number of examples of best practice in the area of MDT at all levels of intervention from participating centres in the study. These included increased involvement of nurse practitioners (Utrecht, the Netherlands), paediatric- and adult psychologist-led consultation and support groups (Sao Paulo, Brazil), and input from pharmacists to boost adherence to topical steroids (Barcelona, Spain). Patients managed in an MDT setting gain better access to diagnostic tests and advice from experts in their field, expedited treatment initiation, and a reduced travel burden. In turn, HCP and healthcare systems benefit from streamlined referrals and

more efficient patient management, improved communications, a reduced burden on resources, less duplication, increased effectiveness, and lower costs. Prof Simpson concluded that MDT involvement is vital for complex patients. This would include a structured and co-ordinated approach that provides holistic patient care and co-ordination between treating HCP and specialists, who serve to streamline and improve patient health management.

Medicine is increasingly focussed on assessing delivered quality of care, noted Prof Simpson, and good-practice Intervention 3 reflects this by monitoring and evaluating care quality. Easy steps towards achieving this include defining care goals, processes, and outcomes; analysis of patient outcomes; and implementation of patient surveys and satisfaction questionnaires. More difficult and advanced approaches could include cross-centre evaluation and HCP assessment, ongoing patient databases, patient dashboards, and external audit of services. Implementing measures to monitor and evaluate care quality gives patients the opportunity to provide direct input that can improve their quality of care, thereby instilling greater confidence in the care provided. HCP benefit from improved patient outcomes, increased patient satisfaction, and better care efficiency, as well as the ability to deliver consistent standards of care. Overall, monitoring and evaluation within centres and amongst wider networks allow for continuous improvement, explained Prof Simpson. Consistent standards of care raise awareness of improvement areas yielding better patient outcomes, patient satisfaction, and care efficiency.

Intervention 4 centres on the important area of patient education and communication, with the aim of improving patient and caregiver understanding of AD and its effective management. Consultant-led patient education, patient intervention plans, referral to patient support groups, and question and answer opportunities are all simple patient education strategies that can be easily applied in daily clinical practice. More difficult and advanced approaches could include intensive educational initiatives, in-house patient support groups and technologies (e.g., bespoke digital apps), patient games and role play, expert patients, and satellite clinics. The benefits of better patient and caregiver education and communication

are obvious, said Prof Simpson, because when patients understand something, adherence is greater and, in turn, outcomes are improved. HCP and healthcare systems therefore benefit from a reduced demand on time, services, and resources. Therefore, overall, better patient involvement in case decisions decreases the burden on patients, HCP, and healthcare systems alike, Prof Simpson concluded.

The final change identified by the Atopic Dermatitis Quality of Care Initiative focusses on the importance of collaboration and exchange with patient groups. The simplest and easiest approach, which can be adopted anywhere, is for all AD treatment centres to routinely direct patients to patient advocacy groups, and vice versa. Prof Simpson emphasised that working collaboratively and exchanging information with patient groups educates and empowers patients and caregivers to actively participate in their AD. Patient access to the resources of patient groups also improves self-management, which in turn decreases clinical and healthcare system burden.

A detailed summary of these key high-priority good practice interventions is contained in the Quality of Care Initiative report.¹

Launching the Global Atopic Dermatitis Quality of Care Initiative

Professor Emma Guttman

Prof Guttman discussed the launch of the Global Atopic Dermatitis Quality of Care Initiative, explaining that the final report from the initiative capturing best practice quality of care from all 32 involved treatment centres is now launched and available online.¹

This interactive website contains all key results from the report, logically presented and fully searchable. Prof Guttman described the report as having very useful applications for everyday clinical practice, in particular for improving clinical care for patients with AD and stepping up treatment where required to maximise QoL and reduce the burden on both patients and wider society. The report also provides the opportunity to compare and contrast clinical practice in AD

from around the world, noted Prof Guttman, allowing clinicians to understand regional differences and embrace examples of good practices wherever they occur.

Discussion

During discussions, all experts agreed that a shift in the landscape of care for AD is urgently needed and that the Global Atopic Dermatitis Quality of Care Initiative will have an important role in delivering this. The availability of the report as a free reference tool accessible by all will also help to raise wider awareness of AD, which panel members highlighted as a key priority. Prof Simpson pointed out that the amalgamated experiences from 32 centres across 17 different countries show that similar clinical challenges in AD are being faced around the world and that the five key strategies outlined in the report can be used successfully to overcome these.

The panel agreed that AD care is entering an exciting era with the availability of improved treatment options but that more needs to be done to optimise outcomes from this expanding therapeutic armoury. Prof Guttman explained that а common misconception amongst patients, fuelled by short-duration clinical trials, is that AD is not a chronic disease and that symptoms will resolve, and treatment can be stopped within a set timeframe (6-12 weeks). Another challenge is that patients with very severe AD can tolerate a high disease burden. so even a slight improvement in symptoms may be seen as treatment success. There is a need to educate patients on these critical issues, said Prof Guttman, to ensure that adherence is maintained over the long term and treatment outcomes improve. On the subject of patient education, experts agreed that this is particularly important at the outset of the patient journey, when it is vital to outline good practices and treatment expectations. Prof Deleuran suggested that it was worth investing more time in education upfront and adopting a holistic approach involving nurses, dietitians, and other members of the MDT to improve overall outcomes for patients. Enhanced collaboration with patient groups and patient organisations was also highlighted as a key focus area by the panel, who emphasised the importance of ensuring teenage and adult patients are not overlooked. "As dermatologists, it is essential

that we partner with patient organisations," stressed Prof Guttman, "because, ultimately, to bring new drugs to patients we need to listen to the patients' voices and understand the direction they want to go in."

Considering comorbidities of AD, in particular the mental health impact, Prof Guttman explained that patients with depression can experience improvements in their depressive symptoms when they receive effective systemic AD therapies, especially if they present with severe AD that affects multiple aspects of their life. Therefore, it is important to understand that some of the comorbidities of AD are induced by the severity of the disease and once the condition is well controlled, these can be minimised or even resolved. Prof Simpson agreed that use of more aggressive therapy can often mitigate the impact of comorbidities on patients, in particular the mental health effects that can be directly attributed to the severity of skin disease.

All panel members acknowledged that primary care doctors currently have the biggest role in treating AD in the USA and Europe because of a lack of dermatologists. A key priority for specialists is therefore to educate general practitioners so that they can better help patients with AD in the primary care system. severity assessment, rather than Disease diagnosis per se, represents one of the biggest challenges currently faced in AD management. It needs to be emphasised that, irrespective of time constraints, assessing the full body surface area is key, said Prof Guttman. This is the single most important factor that indicates the need to step-up treatment from topicals. Prof Simpson concurred, explaining that by making better therapeutic decisions for patients, QoL will be significantly improved and ultimately lead to a cost burden reduction for the healthcare system if patients are well controlled.

Drawing the symposium to an end, panel members issued a call to colleagues around the world to access and read the Global Atopic Dermatitis Quality of Care Initiative report. Even those who are considered experts in AD can learn something from this initiative, concluded Prof Guttman, who stated that there is always scope to evolve and improve our practices to help all AD patients globally benefit from the best care possible.

References

 KPMG International Limited. Improving quality of care in atopic dermatitis. 2020. Available at: http:// atopicdermatitiscare.kpmg.co.uk. Last accessed: 16 December 2020.

2. Bos B et al. Corticosteroid phobia (corticophobia) in parents of young

children with atopic dermatitis and their health care providers. Pediatr Dermatol. 2019;36(1):100-4.



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AML, acute myeloid leukemia; HR-MDS, higher-risk myelodysplastic syndrome; TIM-3, T cell immunoglobulin and mucin domain-3.

References: 1. Wang C, Yang Y, Gao S, et al. Immune dysregulation in myelodysplastic syndrome: clinical features, pathogenesis and therapeutic strategies. *Crit Rev Oncol Hematol.* 2018;122:123-132. **2.** Anderson AC, Joller N, Kuchroo VK. Lag-3, Tim-3, and TIGIT: co-inhibitory receptors with specialized functions in immune regulation. *Immunity.* 2016;44(5):989-1004. **3.** Kikushige Y, Shima T, Takayanagi S-I, et al. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell.* 2010;7(6):708-717.



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

These poster presentations took place between 15th and 17th May 2021, as part of the American College of Cardiology (ACC) Virtual Conference

Presenters:	John Spertus, ¹ John Teerlink ²
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Meeting Summary

Two late-breaking abstracts at the American College of Cardiology's (ACC) virtual meeting ACC.21 delved further into the data of two pivotal trials, demonstrating the additional benefits of new cardiac treatments for obstructive hypertrophic cardiomyopathy (HCM), heart failure (HF), and heart failure with preserved ejection fraction (HFpEF).

John Spertus, consultant cardiologist and director of cardiovascular education and outcomes research at Saint Luke's Hospital, Kansas City, Missouri, USA, presented an analysis of health status data collected during the EXPLORER-HCM randomised clinical trial of mavacamten in obstructive HCM.

Consultant cardiologist and director of heart failure and clinical echocardiography at San Francisco Veterans Affairs Medical Center, San Francisco, California, USA, John Teerlink, spoke about a secondary analysis of data from the GALACTIC-HF trial. His team evaluated the modifying effect of baseline ejection fraction (EF) on the treatment effect of omecamtiv mecarbil.

Results from the EXPLORER-HCM Randomised Clinical Trial: Health Status Benefits of Mavacamten in Patients with Symptomatic Obstructive Hypertrophic Cardiomyopathy

John Spertus

In the EXPLORER-HCM trial, mavacamten, novel direct myosin inhibitor, met its а primary endpoints of improving peak oxygen consumption (pVO₂), and New York Heart Association (NYHA) classification in obstructive HCM.¹ The trial's secondary endpoints assessed changes in post-exercise left ventricular outflow tract (LVOT) gradient, pVO₂, NYHA class, Kansas City Cardiomyopathy Questionnaire (KCCQ), and Hypertrophic Cardiomyopathy Symptom Questionnaire Shortness-of-Breath subscore (HCMSQ-SoB).

Spertus presented the results of a secondary analysis of the results, which sought to better understand the treatment's impact on health status.

Explaining the background, he said that HCM primary myocardial was а disorder of unexplained left ventricular hypertrophy, often caused by pathogenic variants in sarcomeric genes. Current treatment guidelines focus on improving symptoms and function with β-blockers, verapamil, and disopyramide; more invasive options are reserved for those with refractory symptoms. Mavacamten works by targeting the underlying pathophysiology of HCM and reducing the number of excessive myosin-actin cross-bridges in the sarcomere.

During the EXPLORER-HCM trial,¹ 251 patients were randomised to either escalating doses of mavacamten (2.5, 5.0, 10.0, or 15.0 mg per day), or to placebo for 30 weeks. They were reassessed 8 weeks after end of treatment (Week 38 endof-study). Ninety-two per cent of the patients were on HCM monotherapy with β -blockers or calcium channel blockers. Just four people in the treatment group and 16 in the placebo group were not on any background treatment. To provide a more complete understanding of the benefit from the patients' perspective, Spertus went on, quality of life was assessed prior to randomisation, at the 30-week end-of-treatment point and again at Week 38 end-of-study.

Researchers used the 23-item, disease-specific KCCQ, which explicitly asks patients about their symptoms, physical function, social function, and quality of life. KCCQ overall summary scores (KCCQ-OS) range from zero to 100, with higher scores indicating fewer symptoms, better function, and higher quality of life.

As patients with HCM may not necessarily consider themselves to have HF, Spertus said they took part in cognitive debriefing interviews, during which the study team confirmed the relevance and understandability of KCCQ.

Changes from baseline KCCQ-OS were plotted, using the means and standard errors, over each assessment. The primary analysis focussed on the differences between the treatment and placebo groups at 30 weeks. A responder analysis was also performed to inform the observed mean differences.

Thresholds for responder analysis were defined as:

- > ≤-5 points: worsened
- > -5 to <5 points: unchanged
- > 5 to <10 points: small improvement
- > 10 to <20 points: moderate to large improvement
- > \geq 20 points: large to very large improvement.

Results

"The baseline demographics show great comparability between the treatment and placebo groups, with mean KCCQ-OS scores of 66-plus suggesting a moderately significantly impacted population of patients," said Spertus.

The primary results demonstrated a "very early separation" between the mavacamten-treated patients and the placebo-treated patients. This was observed as soon as 6 weeks and maintained at 30 weeks. At the end of 30 weeks, there was a 9.1-point greater change in KCCQ-OS among mavacamten-treated patients than placebotreated patients (Figure 1).



Figure 1: Mean change in KCCQ-OS.

CI: confidence interval; KCCQ-OS: Kansas City Cardiomyopathy Questionnaire-Overall Summary Score.



Figure 2: Percentage of participants who changed by clinically important amounts at 30 weeks.

Approx.: approximately; KCCQ-OS: Kansas City Cardiomyopathy Questionnaire-Overall Summary Score; NNT: number needed to treat.

"That was highly statistically and clinically significant," said Spertus. The team also looked at what happened after the drug was stopped at Week 30. "What we found was when patients came off of treatment, this benefit immediately dissipated. The health status of the mavacamten-treated patients returned to baseline and was no different than the placebotreated patients," said Spertus. The responder analysis looked at the proportion of patients who got much better and those who got worse. Spertus said: "There was a much larger proportion of placebo patients who got worse over 30 weeks, 23% versus 9% as compared with mavacamten. Most impressively, when you look at the patients who derived a very large improvement from treatment, 36% of the mavacamten-treated patients got substantially better, compared to only 15% of the placebo patients. That 21% absolute difference results in a number-needed-to-treat of only about a five, which is a very large benefit in the health status of patients treated with mavacamten." (Figure 2).

Highlighting the limitations of the study, Spertus said this was a short-term trial, so much research is needed before clinicians can be sure of the long-term impact of mavacamten treatment on health status. In addition, there were some missing data; however, the team conducted extensive analyses, and found no observable biases.

Conclusions

Summing up, Spertus said mavacamten was associated with substantial health status improvements in patients with symptomatic obstructive HCM, and that clinicians would only need to treat five people to achieve one improvement of >20 points as measured by KCCQ-OS. "These benefits were observed early after initiating treatment, and they regressed when treatment was stopped."

Secondary Analysis from GALACTIC-HF: Impact of Ejection Fraction on the Therapeutic Effect of Omecamtiv Mecarbil in Patients with Heart Failure and Reduced Ejection Fraction

John Teerlink

The Global Approach to Lowering Adverse Cardiac outcomes Through Improving Contractility in Heart Failure (GALACTIC-HF) trial achieved its primary endpoint of reducing the risk of cardiovascular death in patients with HF and reduced EF or HF with preserved ejection fraction (HFpEF).²

"But given its mechanism of increasing cardiac function, and based on prespecified subgroup analyses, we assessed the modifying effect of the baseline EF on the beneficial treatment effect of omecamtiv mecarbil," said Teerlink.

GALACTIC-HF

GALACTIC-HF enrolled patients with symptomatic chronic HF with EF of \leq 35%. A total of 8,256 patients were randomised to either omecamtiv mecarbil or placebo, in addition to their usual HF therapy.² Teerlink described it as "one of the broadest ranges of patients enrolled in a HFpEF trial to date."

"It was a positive trial, significantly reducing the primary composite endpoint of time to first HF event or cardiovascular death by 8%," said Teerlink. "For the first time, these results confirm the hypothesis that selectively increasing cardiac function with something such as omecamtiv mecarbil could actually improve clinical outcomes in patients with HFpEF."

"Within the data, those with more severe HF appeared to have a greater benefit," he went on.

Secondary Analysis

Teerlink and his team carried out а analysis to investigate the secondary modifying effect of EF on the beneficial treatment effect of omecamtiv mecarbil. While all GALACTIC-HF patients had an EF of \leq 35% at baseline, >70% had an EF of \leq 30%. Those in the highest quartile (Q4) had an EF of ≥33%.

Patients in the lowest quartile (Q1) had an EF of ≤22%. As well as a lower prevalence of multiple cardiovascular conditions, more nonischaemic cardiomyopathy, and lower BMI, these patients also had greater use of angiotensin receptor neprilysin inhibitors (ARNi), digoxin, ivabradine, and device therapy.

"The primary composite endpoint was the time to first HF event or cardiovascular death," he explained. "Despite excellent baseline HF therapies, 20-50% of the patients in the placebo group had an endpoint event within one year, with the rate markedly increasing with decreasing EF [...] Those in the omecamtiv mecarbil group also had an increasing event rate with decreasing EF, ranging from approximately 22% to 32%."

In the omecamtiv mecarbil group, there was a progressive beneficial reduction in the absolute event rate with decreasing EF. Treatment also decreased the relative risk of the primary endpoint and had a greater relative **Conclusions** treatment effect in patients with lower EF.

With respect to other variables and events of interest, Teerlink said there was no significant difference in systolic blood pressure, serum potassium, or creatinine between the two groups. In addition, serious adverse events and adjudicated arrhythmic and ischaemic event rates were also similar.

In conclusion, Teerlink said that the drug appeared safe, and that fewer than 12 patients would need to be treated to prevent one HF event or cardiovascular death. "Thus, omecamtiv mecarbil represents a novel therapy that holds the promise of improving clinical outcomes in patients with severely reduced EF; the very patients who were the most challenging for us to treat," he said.

References

1. Olivotto I et al. Mavacamten for treatment of symptomatic obstructive hypertrophic cardiomyopathy (EXPLORER-HCM): a randomised, double-blind, placebocontrolled, Phase 3 trial. Lancet. 2020;396(10253):759-69.

2. Teerlink R et al. Cardiac myosin activation with omecamtiv mecarbil in systolic heart failure. N Engl J Med. 2021:384:105-16.

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Relating Ventilatory Support and Drug Treatment Strategies to the Fundamental Pathophysiology in COVID-19 Illness

The Editor's Pick for this issue is the excellent review by Lewis et al., which discusses the current pathophysiologic and radiologic findings related to the different coronavirus disease (COVID-19) stages, as well as mechanisms behind alveolar epithelial damage caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). An improved understanding of the nature of this damage is paramount for better informing targeted pharmacotherapies and ventilatory support.

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Abstract

This article relates the current pathophysiologic and radiologic findings to the fundamental idea of acute lung epithelial infection, alveolar inflammation causing leak into the interstitial space, and subsequent secondary or concurrent endothelial infection and dysfunction. Understanding the mechanisms and timings of alveolar damage can better inform the types of ventilatory support required and timing of targeted pharmacotherapies.

INTRODUCTION

Coronavirus disease (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2);¹ as of September 2020, there were >33 million cases and almost 1 million deaths worldwide.² An unknown number of people are asymptomatic carriers of SARS-CoV-2 but symptoms in the early phase of viral replication include fatigue, anosmia, fever, sore throat, and cough. Around 85–95% of people make an uncomplicated recovery within 10 days; however, some individuals develop worsening cough, dyspnoea, and persistent (higher) fever, some of whom require hospital admission with hypoxia and interstitial pneumonia.³ This more severe illness is from viral infection within the lung tissue and the associated immune dysfunction, with loss of regulation of the inflammatory cascade leading to further lung damage, worsening hypoxia, and other organ dysfunction.

FUNDAMENTAL PATHOPHYSIOLOGY

Two possible inflammation mechanisms (not mutually exclusive) can result in fluid accumulation in the lung alveoli: 1) direct viral and immune-mediated damage to the epithelium, affecting alveolar movement and reducing pulmonary fluid clearance; 2) a secondary or parallel injury to the endothelium or its basement membrane that results in capillaritis with further leak of fluid from the plasma into the interstitial space and thereon into the air spaces.

IMPACT OF EPITHELIAL DAMAGE

SARS-CoV-2 targets and damages pulmonary epithelium,⁴ particularly ciliated airway epithelial cells and Type 2 pneumocytes through the abundant angiotensin-converting enzyme 2 receptors in the lung. The virus relies on cell membrane serine protease TMPRSS2 cleaving and subsequently activating S-proteins and furine protease activity.⁵ Hydrostatic and osmotic forces determine fluid movement from the vascular space to the interstitium through the extracellular matrix and the endothelial glycocalyx layer. Once the normally tight alveolar epithelial barrier is breached, alveolar oedema ensues. The epithelium also performs an important function in reabsorption of fluid through the asymmetric distribution of sodium channels and pumps throughout the alveoli and small airways. Sodium from the alveolar fluid passively enters the apical part of epithelial cells via the sodium channel and is pumped out of the cell via a sodium-potassium pump on the basal membrane. This shift of sodium causes an osmotic gradient leading to movement of water out of the alveoli, avoiding or resolving oedema in the alveolar air space (Figure 1).⁶ Inflammatory damage that increases permeability of the epithelial barrier or a reduction in the number of epithelial cells, channels, and/ or pumps would therefore impair an important clearance mechanism that helps avoid or resolve

pulmonary oedema. Hypoxia itself is extremely proinflammatory and can further exacerbate tissue damage through cytokine release in a selfperpetuating and rapidly escalating autoimmune cycle of harm. Hypoxia further attenuates the basal pumps' highly energy-dependent sodiumpotassium pump clearance mechanism.

POTENTIAL VENTILATION OR VENTILATOR DAMAGE TO THE PULMONARY ENDOTHELIUM OR EPITHELIUM

Normal breathing at tidal volume can further stretch damaged and friable epithelium or endothelium and increase the permeability further. Excessive movements of damaged alveolar units through mechanical stress or sheer stress exacerbate extravasation of fluid. worsening hypoxia. In this regard, the authors propose that ventilatory support strategies should allow enough alveolar capillary oxygenation with a minimal degree of alveolar movement. Reducing physical movement of a leaky epithelium would reduce leak, attenuate further hypoxia, and buy more time for the body to clear the virus or for the antivirals to take effect, and for timely, targeted immunosuppression to reduce bystander cellular damage.

EVIDENCE FROM PULMONARY IMAGING

CT chest scans report ground-glass opacities, often together with areas of consolidation and lung interlobular septal thickening, predominantly in subpleural and middle/lower lung fields.⁷⁻¹⁰ Plain chest radiographs show multiple patchy shadows,^{7,8,11} and ultrasounds report B-lines ranging "across a continuum from mild alveolar interstitial pattern, severe bilateral infiltration pattern to lung consolidation".¹²

Serial CT scans show early subpleural bilateral patchy focal infiltrates, which coalesce into large confluent patchy areas, becoming geographically connected in a 'crazy-paving' pattern and ultimately progressing to diffuse lung consolidation.^{13,14} These sequential images represent progressive areas of alveolar epithelial damage, consistent with a spreading infection and local associated immune reaction.





AEC: alveolar epithelial cells; Na: sodium.

This patchy distribution supports an aerogenous route of pulmonary infection.¹⁵ CT scans usually correlate with clinical severity,⁷ but unlike traditional models of acute respiratory distress syndrome (ARDS) there can be severe radiological changes in relatively well and even asymptomatic people. Conversely, some symptomatic patients may present with a normal CT, especially in the early phase.¹⁶

Imaging in acute COVID-19 is entirely consistent with patchy progressive infection and distal alveolar inflammatory leak.¹² Pleural effusions are rare in COVID-19 because the leak into the third space is not due to hydrostatic back-pressure, but due to small multifocal areas of patchy alveolar epithelial and endothelial damage.^{7,9}

EVIDENCE FROM GROSS LUNG PATHOLOGY

Gross thoracic changes include uneven mottled red to blue-red colour, increased lung weight, oedema, severe congestion, pleuritis, consolidation thrombi, and often secondary bacterial infection.^{17,18}

EVIDENCE FROM LUNG HISTOPATHOLOGY

Antemortem histopathological changes were seen coincidentally in samples of lung tissue taken for other reasons. Findings included alveolar oedema, fibrinous exudate, inspissated protein, haemorrhage, mononuclear inflammation, and multinucleated giant cell formation.^{18,19} Two patients developed severe COVID-19, and one of these patients died.²⁰

Postmortem histopathological changes included desquamation of the alveolar epithelium, reactive Type 2 pneumocyte hyperplasia, intra-alveolar exudates with organising fibrin, hyaline membrane formation, haemorrhage, excess lymphocytes, neutrophils, alveolar megakaryocytes, thrombosis of small and midsized pulmonary arteries, and some interstitial fibrosis.^{14,21-23} From the autopsies of 21 individuals who died as a result of COVID-19, the primary cause of death was reported as respiratory failure, with exudative diffuse alveolar damage, and with severe capillary congestion and microthrombi in alveolar capillaries. Pulmonary embolism (n=4), alveolar haemorrhage (n=3), vasculitis (n=1), and superimposed bronchopneumonia (n=10) were also reported.¹⁸

The inflammation is predominantly lymphocytic with large atypical pneumocytes,¹¹ probably caused by viral cytopathic changes.^{11,18,24} Macrophages and monocytes are increased in alveolar spaces, and virus particles or antigens are detected in the epithelial cells, Type 1 and 2 pneumocytes, and macrophages by electron microscopy, immunohistochemistry, and *in situ* hybridisation.²¹

The primary damage occurs mainly to the alveolar epithelial cells. Initially, some research proposed that the lack of direct damage to endothelial cells may partly explain why other organs (heart: 7–9%; kidney: 3–15%) are less affected than the lungs.²⁵⁻²⁸ However, COVID-19 clearly incorporates other organ dysfunction and much more so than in other coronaviruses (severe acute respiratory syndrome and Middle East respiratory syndrome) and influenza.²⁹

Endothelial cell viral infection, inflammatory cell infiltration, apoptosis of endothelial and mononuclear cells with widespread thrombosis,³⁰ microangiopathy, marked increase in fibrinous microthrombi, and both intussusceptive and conventional sprouting angiogenesis³¹ have been reported. In one systematic pulmonary dissection study, all patients exhibited thrombosis of small or midsized pulmonary

arteries.²² Here, the authors postulate that direct infection of endothelial cells does occur and their close proximity to the epithelium in the lung is why the lung is affected most by local vascular dysregulation,^{22,30,32} leading to further hypoxia without loss of lung compliance in the early phase.

EVIDENCE FROM PATHOPHYSIOLOGY

Intubated Patients

Standard ARDS Berlin criteria require an onset within 7 days of a clinical insult or respiratory symptoms;³³ however, in COVID-19, respiratory failure occurs typically 10-15 days after onset of symptoms.²⁵⁻²⁸ Early cohorts had worse outcomes with over-inflation^{34,35} and higher levels of barotrauma compared to ventilated with standard ARDS.35 Current patients COVID-19 ventilator guidelines consistently recommend lower pressures, probably to reduce alveolar stretch.^{36,37}

Unlike most people with ARDS, many patients with COVID-19 receiving ventilation (especially early on) have compliant lungs despite profound oxygenation failure.^{38,39} Some studies have defined separate phenotypes in COVID-19 pneumonitis according to lung compliance,⁴⁰ but whether these are truly distinct or sequential stages of the same disease is in debate. Relatively high compliance is associated with high shunt fractions (50%), rarely seen in other forms of ARDS.⁴⁰ Again, this supports the idea that disrupted vasoregulation is an important contributor to poor oxygenation, at least in the early stages of COVID-19 lung injury. A major problem is the failure of the normal hypoxiainduced pulmonary vasoconstriction,⁴¹ which depends on good endothelial function, leading to hyperperfusion of 'gasless tissue'. CT scans report areas of dilated vessels in parts of the lung that are unlikely to be well ventilated.^{10,41} The improvement of oxygenation with positive end-expiratory pressure and prone positioning may be due to haemodynamic adjustment through local fluid shifts and better alveolar recruitment.³⁹

The origin of the 'preserved' compliance is unknown; while it is probably beneficial for ventilation, it may have other detrimental effects. The lung possesses a highly efficient mechanism to minimise interstitial fluid volume. The pulmonary capillary basement membrane normally has low permeability to water due to the macromolecular organisation of heparan sulfate proteoglycans. Furthermore, any increase in extravascular fluid is rapidly cleared because of the high elastance (poor compliance) of the extracellular matrix consisting of a second group of matrix chondroitin sulfate proteoglycans. Hypoxia causes fragmentation of both sets of proteoglycans, resulting in decreased elastance (increased compliance) and leading to more alveolar movement and potential oedema.42 Applying high levels of positive end-expiratory pressure may accentuate underlying alveolar and microvascular injury and contribute to a worse outcome.43

The authors suggest that the respiratory distress seen in COVID-19 starts with a primary epithelial failure, followed by a secondary or concurrent endothelial vascular dysfunction. Therefore, ventilation should be guided by daily changes in radiology and estimates of capillary wedge pressure and lung water. Cardiac dysfunction viral-induced from myocarditis and/or hypoxaemia, worsening any pre-existing cardiac conditions, may also complicate vascular filling. Various fluid balance strategies have been tried in typical ARDS, based on estimated pulmonary capillary wedge pressure and extravascular lung water, but the unusual pulmonary haemodynamics seen in COVID-19 are a further complicating factor in the cardiac-pulmonary-specific interactions in intubated patients.

The wide variations in mortality of intubated patients with COVID-19^{26,38,39} could be due to fixed ventilatory strategies and basing pressure or target volumes on traditional ARDS models. The authors argue that the traditional (Berlin) definitions of ARDS do not align with COVID-19 respiratory failure in terms of diagnosis or, more importantly, ventilator management of lower pressures and daily changes according to variable haemodynamics and hypoxaemia. Extracorporeal membrane oxygenation (ECMO) is increasingly used for adult patients with potentially reversible respiratory failure refractory to conventional management. ECMO beds have been increased worldwide in response to the pandemic and some countries have issued guidelines to assist in identifying

eligible patients and management.⁴⁴ However, most clinicians in the ECMO service agree that the current evidence base does not allow strict criteria to determine optimal benefit and therefore encourage early referral. In reality, the experience (of the authors) has been that decisions are often made according to ECMO availability.

Awake Patients

Hypoxic patients with COVID-19 are commonly seen taking rapid and shallow breaths.45 Increased pulmonary interstitial pressure or volume activates pulmonary afferent C-fibre receptors, which are known to initiate a reflex that includes rapid shallow breathing and dyspnoea.⁴⁶ These receptors are stimulated by increases in pulmonary interstitial pressure or volume (as occurs in pulmonary oedema). The combination of hypoxia, hypocapnia, tachypnoea and pulmonary oedema as seen in altitude sickness,⁴⁷ traumatic 'blast lung',48 and chemical lung injury⁴⁹ are explained by the increased minute ventilation in response to the hypoxia; there is a disproportionate elimination of CO₂ because its exchange is not impaired by pulmonary oedema as much as oxygen.

Awake patients with COVID-19 tolerate extreme hypoxia surprisingly well. Many patients converse freely despite peripheral oxygen saturations of 80–92% but have respiratory rates of 30– 38 breaths per minute, suggesting that rapid, shallow breathing is a natural response. Deeper inspiration causes dyspnoea and often back or chest pain in individuals with radiological COVID-19 pneumonitis (personal observation from Lewis KE).

However, if patients experience worsening hypoxia and start taking strong, spontaneous inspiratory efforts, this can physically stretch an already damaged alveolar epithelium and increase tissue stresses (akin to patient selfinflicted lung injury).⁵⁰ Coexistent endothelial dysfunction with ventilation/perfusion mismatch leads to rapidly worsening hypoxaemia, more inflammation, more leak, and more hypoxia.

However, one notable difference is that dyspnoea is much less pronounced in COVID-19. Fifteen percent of patients with mild-case COVID-19 had shortness of breath, and only 38% of severe cases in China²⁷ and 17% of hospitalised

cases in New York, USA, with tachypnoea⁵¹ complained of breathlessness. Whether this is of any significance in terms of respiratory control mechanisms remains to be seen.

Patients experiencing mild COVID-19 pneumonitis often have rapid, shallow breathing at rest, with normal arterial oxygen saturation. Frequently in these patients, mild exercise precipitates peripheral oxygen desaturation, consistent with an initial small reduction in pulmonary capillary gas transfer, which is sufficient to activate the pulmonary afferent C-fibre receptors and alter the breathing pattern.⁴⁶ In normal lungs at rest, however, because the blood becomes fully saturated with oxygen by the time it has travelled approximately one-third the length of the pulmonary capillary, there is sufficient reserve to allow nearly complete saturation when the patient is at rest.⁵² However, with mild exercise the increased cardiac output reduces pulmonary blood transit time, so that pulmonary gas transfer now becomes diffusion-limited with consequent arterial desaturation. During recovery and rehabilitation, patients still profoundly desaturate, suggesting that it takes some time for the pulmonary capillary diffusion barriers to completely resolve.

SUPPORTING EVIDENCE FROM CONTINUOUS POSITIVE AIRWAY PRESSURE

Despite early experience with invasive ventilation, many healthcare professionals are moving towards noninvasive ventilation strategies for hypoxic COVID-19.53 Mild-to-moderate oxygenation failure in COVID-19 responds to high-flow nasal oxygen (HFNO) systems or continuous positive airway pressure (CPAP).³ These treatments allow the patient to eat, drink, communicate, and self-position. By reducing the work of breathing and inspiratory effort, it can reduce mechanical sheer stress, ventilationinduced lung injury, tracheostomy, ventilationassociated pneumonia, and difficulty in weaning. CPAP may delay or avoid the need for mechanical ventilation in patients with COVID-19.27,53-55 When intensive treatment unit resources are scarce, inappropriate use of invasive ventilation may stop access to life-saving treatment to those that need it. CPAP is part of the UK recommendations for the management of

COVID-19.^{41,56} It is effective in the treatment of chemical lung injury with pulmonary oedema, reduced gas transfer, and high shunt fraction.⁵⁷

These pathophysiological mechanisms explain how a ventilatory support strategy using CPAP and HFNO maintains both a physical pressure gradient and a partial-pressure gradient to allow continuous oxygen diffusion down the airways and across gently splinted-open alveoli that do not need to move much. This also allows rapid, small breaths to minimise movement of damaged epithelial and endothelial interfaces and supports fatigued muscles. Combining CPAP with HFNO allows maximum diffusion at the alveolar-capillary interface with minimum alveolar movement (Figure 2).

ADDITIONAL IMPORTANT TREATMENTS

Adequate fluid replacement is important as patients are prone to dehydration through increased insensible losses (fever, tachypnoea, occasionally diarrhoea) and reduced oral intake (nausea, CPAP masks).

Prone positioning can help oxygenation in some patients (while awake, on CPAP and ventilators). Presumably, prone positioning helps recruit more or different alveoli and therefore aligns with the hypothesis of localised inflammation and alveolar leak, rather than alveolar oedema from heart failure or increased hydrostatic pressure, which often worsen when lying supine or prone.

Management of coexistent medical conditions, especially diabetes, cardiac dysfunction, pulmonary emboli, and superadded bacterial or fungal lung infections, is also crucial. Worsening symptoms (including new confusion), worsening hypoxia, higher ventilatory pressure support, new raised temperature, rising inflammatory markers (neutrophils and C-reactive protein), raised procalcitonin, and especially focal consolidation and fluid or cavities on chest X-rays or CT scans, should alert clinicians to coincidental/secondary infections. Bacterial and fungal infections are reported in at least 10-30% of postmortem lung specimens of people who died of COVID-19.^{18,21-23}



Figure 2: Combined benefits of airway pressure and oxygen.

CPAP: continuous positive airway pressure; FiO,: fraction of inspired oxygen; PEEP: positive end-expiratory pressure.

SUPPORTING EVIDENCE FROM DRUG TREATMENTS

A detailed review of all current pharmacological treatments is beyond the scope of this article. However, the predominant success so far of immunosuppressant and immunomodulatory drugs fits with the idea of reducing primary inflammatory epithelial/endothelial damage.

The UK multicentre RECOVERY trial showed dexamethasone reduced deaths in ventilated patients (rate ratio: 0.65; 95% confidence interval [CI]: 0.48–0.88; p=0.0003) and deaths in patients receiving oxygen (rate ratio: 0.80; 95% CI: 0.67–0.96; p=0.0021). There was no benefit among those patients not requiring respiratory support (rate ratio: 1.22; 95% CI: 0.86–1.75; p=0.14)⁵⁸ presumably because there was no bystander immune-related damage at this point.

A meta-analysis showed that systemic steroids improve mortality in critically ill patients with COVID-19, likely due to cytokine storms and because steroids reduce bystander immunerelated alveolar damage.⁵⁹ incite cytokine storms with large amounts of IL-6; therefore, monoclonal antibodies that target the IL-6 pathways may potentially curb these inflammatory storms. Tocilizumab, a monoclonal antibody against IL-6, appears to reduce mortality by approximately 24% and duration of organ support by around 1 week,

Pathogenic T cells and inflammatory monocytes



Figure 3: Changes in arterial oxygen saturation (skeletal muscle oxygen saturation, pulse oximetry), RR, and HR, on replacement of CPAP with high-flow oxygen during clinical care (to allow eating and drinking), and subsequent reintroduction of CPAP.

Comments on traces for Patient 3 were taken from audio recording coincident with cardiorespiratory monitoring. CPAP: continuous positive airway pressure; HR: heart rate; RR: respiratory rate; SpO₂: arterial oxygen saturation. in patients in intensive care units who need ventilatory support and have already received steroids (number needed to treat: 12).⁶⁰ Tocilizumab may not prove to be so effective in patients less critically ill and who only require oxygen.⁶¹

Other inflammatory and immunomodulatory drugs blocking endothelial activation and injury⁶² or boosting pulmonary epithelial cell clearance of alveolar oedema (Figure 1) may also be useful.⁶³ Other therapies such as the antiviral drugs remdesivir, lopinavir, hydroxychloroquine, and interferon have not been shown to reduce mortality or initiation of ventilation.⁶⁴ Convalescent plasma and hyperimmune immunoglobulins are still being tested.

CLINICAL CASES

The following clinical cases illustrate the pathophysiology and response to CPAP treatments.

A 59-year-old male was brought to hospital by ambulance. He was mildly overweight and had controlled hypertension. On arrival, his peripheral capillary oxygen saturation (SpO₂) was 67%, despite receiving oxygen 15 L/min via a nonrebreather mask. Arterial blood gases confirmed severe Type 1 respiratory failure (partial pressure of arterial oxygen: 5.3 kPa; partial pressure of CO2: 4.3 kPa). His quick sepsis-related organ failure score was 1 (blood pressure: 130/60 mmHg; respiratory rate: 38 breaths/min; Glasgow coma score: 15) but despite hypoxia he was able to speak on the phone. SpO₂ improved to 80% and respiratory rate reduced to 24 breaths per minute with nasal CPAP (12 cmH₂O pressure) with entrained oxygen at 15 L/min. Improvement in temperature and oxygenation occurred 5 hours after receiving 500 ma of intravenous confirmed bilateral methylprednisolone. CT extensive ground-glass shadowing and confluent infiltrates. A swab was positive for viral RNA of SARS-CoV-2. He was not intubated but continued CPAP with HFNO for 15 days with gradual weaning of both. He received six further

doses of high-dose steroids (pre-RECOVERY results) and was discharged after 21 days.

Figure 3 displays transcripts from real-time videos of ward monitors from Patient 2 (53- year-old male, nonsmoker) and Patient 3 (58-year-old male, nonsmoker). Neither had any known lung or heart disease. Both patients were receiving 15 L/min of oxygen entrained through their CPAP machine (Trilogy Evo, Philips, Amsterdam, the Netherlands) set at 12–15 cmH₂O pressure.

The graphs demonstrate abrupt falls in oxygen saturations and rises in pulse and respiratory rate, because the CPAP mask was removed to allow the patients to drink and take short breaks. Both were immediately switched to oxygen nasal cannulas at 15–20 L/min. Neither felt unduly breathless on removing their masks; however, Patient 3 reported feeling tired. Their SpO₂ and pulse improved within 20 seconds of reapplying the positive pressure, suggesting that combining CPAP with HFNO is beneficial in people where oxygen alone is not sufficiently effective.

CONCLUSION

By relating underlying changes in epithelia, subsequent endothelial infection and dysfunction, and the pathophysiology of alveolar leak, the different disease stages and why certain combinations of treatments are most effective can begin to be understood. More importantly, the rapid increase in knowledge, based initially on observational physiology and now interventions, supports the concept of sequential epithelial and endothelial failure and explains differences in individuals over time and the variable response to treatments. Most importantly, understanding why and how pathophysiology changes so quickly allows for tailored interventions. Appropriate selection of early CPAP and timely immunosuppression should further improve outcomes. Certainly, one (treatment) size does not fit all, and one size will not fit one person over time.

References

- Alhazzani W et al. Surviving Sepsis Campaign: guidelines on the management of critically ill adults with coronavirus disease 2019 (COVID-19). Crit Care Med. 2020;48(6):e440-69.
- World Health Organization (WHO). WHO coronavirus disease (COVID-19) dashboard. Available at: https:// covid19.who.int. Last accessed: 9 February 2021.
- Giwa AL et al. Novel 2019 coronavirus SARS-CoV-2 (COVID-19): an updated overview for emergency clinicians. Emerg Med Pract. 2020;22(5):1-28.
- Bao L et al. The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice. Nature. 2020;583:1-20.
- Hoffmann M et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271-8.
- Matthay MA et al. Lung epithelial fluid transport and the resolution of pulmonary edema. Physiol Rev. 2002;82:569-600.
- Wu J et al. Chest CT findings in patients with coronavirus disease 2019 and its relationship with clinical features. Invest Radiol. 2020;55:257-61.
- Schaller T et al. Postmortem examination of patients with COVID-19. JAMA. 2020;323(24):2518-20.
- 9. Shi H et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020;20:425-34.
- Zhao W et al. Relation between chest CT findings and clinical conditions of coronavirus disease (COVID-19) pneumonia: a multicenter study. AJR Am J Roentgenol. 2020;214(5):1072-7.
- Xu Z et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020;8:420-2.
- Peng Q-Y et al. Findings of lung ultrasonography of novel corona virus pneumonia during the 2019–2020 epidemic. Intensive Care Med. 2020;46(5):849-50.
- Pan F et al. Time course of lung changes at chest CT during recovery from coronavirus disease 2019 (COVID-19). Radiology. 2020;295:715-21.
- National Health Service (NHS) Health Education England. East of England School of Radiology. Available at: https://heeoe.hee.nhs.uk/radiology. Last accessed: 9 February 2021.
- Hou YJ et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. Cell. 2020;182(2):429-46.e14.

- Kanne JP et al. Essentials for radiologists on COVID-19: an updateradiology scientific expert panel. Radiology. 2020;296(2):E113-E114.
- Buja LM et al. The emerging spectrum of cardiopulmonary pathology of the coronavirus disease 2019 (COVID-19): report of 3 autopsies from Houston, Texas, and review of autopsy findings from other United States cities. Cardiovasc Pathol. 2020;48:107233.
- Menter T et al. Post-mortem examination of COVID19 patients reveals diffuse alveolar damage with severe capillary congestion and variegated findings of lungs and other organs suggesting vascular dysfunction. Histopathology. 2020;77(2):198-209.
- Pernazza A et al. Early histologic findings of pulmonary SARS-CoV-2 infection detected in a surgical specimen. Virchows Arch. 2020;477(5):743-8.
- Tian S, Xiao S-Y. Pathology of 2019 novel coronavirus pneumonia: a dynamic disease process. J Thorac Oncol. 2020;15(5):e67-8.
- Zhang H et al. Histopathologic changes and SARS-CoV-2 immunostaining in the lung of a patient with COVID-19. Ann Intern Med. 2020;172(9):629-32.
- 22. Lax SF et al. Pulmonary arterial thrombosis in COVID-19 with fatal outcome: results from a prospective, single-center, clinicopathologic case series. Ann Intern Med. 2020;173(5):350-61.
- Thüsen von der J, van der Eerden M. Histopathology and genetic susceptibility in COVID-19 pneumonia. Eur J Clin Invest. 2020;50(7):e13259.
- Tian S et al. Pathological study of the 2019 novel coronavirus disease (COVID-19) through postmortem core biopsies. Mod Pathol. 2020;33(6):1007-14.
- Chen N et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020;395:507-13.
- 26. Wang D et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA. 2020;323:1061-9.
- Guan W-J et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382:1708-20.
- 28. Zhou F et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395:1054-62.
- 29. Pons S et al. The vascular endothelium: the cornerstone

of organ dysfunction in severe SARS-CoV-2 infection. Crit Care. 2020;24:353-8.

- Varga Z et al. Endothelial cell infection and endotheliitis in COVID-19. Lancet. 2020;395:1417-8.
- Ackermann M et al. Pulmonary vascular endothelialitis, thrombosis, and angiogenesis in COVID-19. N Engl J Med. 2020;383:120-8.
- 32. Kaur S et al. The enigma of endothelium in COVID-19. Front Physiol. 2020;11:989.
- ARDS Definition Task Force; Ranieri VM et al. Acute respiratory distress syndrome: the Berlin definition. JAMA. 2012;307(23):2526-33.
- Liu X et al. Ventilatory ratio in hypercapnic mechanically ventilated patients with COVID-19 associated ARDS. Am J Respir Crit Care Med. 2020;201(10):1297-9.
- McGuinness G et al. High incidence of barotrauma in patients with COVID-19 infection on invasive mechanical ventilation. Radiology. 2020;297(2):E252-62.
- Roesthuis L et al. Advanced respiratory monitoring in COVID-19 patients: use less PEEP! Crit Care. 2020;24:230-4.
- National Health Service (NHS). Clinical guide for the management of critical care for adults with COVID-19 during the coronavirus pandemic. Available at: https://www.nice.org. uk/Media/Default/About/COVID-19/ Specialty-guides/Specialty-guide_ Adult-critical-care.pdf. Last accessed: 9 February 2021.
- Grasselli G et al. Baseline characteristics and outcomes of 1591 patients infected with SARS-CoV-2 admitted to ICUs of the Lombardy region, Italy. JAMA. 2020;323:1574.
- 39. Arentz M et al. Characteristics and outcomes of 21 critically ill patients with COVID-19 in Washington state. JAMA. 2020;323:1612-4.
- Gattinoni L et al. Covid-19 does not lead to a "typical" acute respiratory distress syndrome. Am J Respir Crit Care Med. 2020;201(10):1299-300.
- Pfeifer M et al. [Position paper for the state of the art application of respiratory support in patients with COVID-19 - German Respiratory Society]. Pneumologie. 2020;74(6):337-57. (In German).
- 42. Miserocchi G. Lung interstitial pressure and structure in acute hypoxia. Adv Exp Med Biol. 2007;618:141-57.
- 43. Marini JJ, Gattinoni L. Management of COVID-19 respiratory distress. JAMA. 2020;323(22):2329-30.
- 44. Shekar K et al. Extracorporeal Life Support Organization

Coronavirus Disease 2019 Interim Guidelines: a consensus document from an international group of interdisciplinary extracorporeal membrane oxygenation providers. ASAIO J. 2020;66(7):707-21.

- 45. Bennett J et al. Respiratory advice for the non-respiratory physician in the time of COVID-19. Clin Med. 2020;20:251-5.
- Paintal AS. Mechanism of stimulation of Type J pulmonary receptors. J Physiol. 1969;203:511-32.
- 47. Solaimanzadeh I. Acetazolamide, nifedipine and phosphodiesterase inhibitors: rationale for their utilization as adjunctive countermeasures in the treatment of coronavirus disease 2019 (COVID-19). Cureus. 2020;12:e7343.
- Kirkman E, Watts S. Characterization of the response to primary blast injury. Philos Trans R Soc Lond B Biol Sci. 2011;366(1562):286-90.
- 49. Jugg BJA et al. The injured lung: clinical issues and experimental models. Philos Trans R Soc Lond B Biol Sci. 2011;366(1562):306-9.
- 50. Brochard L et al. Mechanical ventilation to minimize progression of lung injury in acute respiratory failure. Am J Respir Crit Care Med. 2017;195:438-42.
- 51. Richardson S et al. Presenting characteristics, comorbidities, and outcomes among 5700 patients hospitalized with COVID-19 in

the New York City area. JAMA. 2020;323:2052-9.

- Wagner PD, West JB. Effects of diffusion impairment on O₂ and CO₂ time courses in pulmonary capillaries. J Appl Physiol. 1972;33:62-71.
- Arulkumaran N et al. Use of noninvasive ventilation for patients with COVID-19: a cause for concern? Lancet Respir Med. 2020;8(6):e45.
- 54. Nightingale R et al. Is continuous positive airway pressure (CPAP) a new standard of care for Type 1 respiratory failure in COVID-19 patients? A retrospective observational study of a dedicated COVID-19 CPAP service. BMJ Open Respir Res. 2020;7(1):e000639.
- 55. Villarreal-Fernandez E et al. A plea for avoiding systematic intubation in severely hypoxemic patients with COVID-19-associated respiratory failure. Crit Care. 2020;24(1):337.
- 56. National Health Service (NHS). Guidance for the role and use of noninvasive respiratory support in adult patients with COVID-19 (confirmed or suspected). Available at: https://www. nice.org.uk/Media/Default/About/ COVID-19/Specialty-guides/specialtyguide-NIV-respiratory-supportand-coronavirus.pdf. Last access: 9 February 2021.
- Graham S et al. Continuous positive airway pressure: an early intervention to prevent phosgene-induced acute lung injury. Toxicol Lett. 2018;293:120-6.

- RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with COVID-19 preliminary report. N Engl J Med. 2020;DOI:10.1056/NEJMoa2021436.
- 59. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group; Sterne JAC et al. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: a meta-analysis. JAMA. 2020;324:1330-41.
- 60. The REMAP-CAP Investigators; Gordon AC et al. Interleukin-6 receptor antagonists in critically ill patients with COVID-19 - preliminary report. medRxiv. 2021;DOI:10.1101/202 1.01.07.21249390. [Preprint].
- 61. Stone JH et al. Efficacy of tocilizumab in patients hospitalized with COVID-19. N Engl J Med. 2020;383:2333-44.
- 62. Simonsen U et al. Emerging roles of calcium-activated K channels and TRPV4 channels in lung oedema and pulmonary circulatory collapse. Acta Physiol. 2017;219:176-87.
- He J et al. Rosiglitazone promotes ENaC-mediated alveolar fluid clearance in acute lung injury through the PPARγ/SGK1 signaling pathway. Cell Mol Biol Lett. 2019;24:35.
- 64. WHO Solidarity Trial Consortium; Pan H et al. Repurposed antiviral drugs for COVID-19 - interim WHO solidarity trial results. N Engl J Med. 2020;NEJMoa2023184.



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

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Abstract

Non-dystrophic myotonias (NDM) manifest as delayed muscle relaxation leading to muscle stiffness. This may diminish or worsen with repeated contractions, depending on NDM subtype. These are divided into those affecting the chloride channel CLC-1, due to *CLCN1* gene mutations, and those affecting the sodium channel NaV1.4, due to *SCN4A* gene mutations. Depending on NDM subtype,
additional symptoms and clinical signs of NDM can include transient weakness, myalgia, cramps, fatigue, dysphagia, dysphonia, and muscle hypertrophy. Two surveys, carried out independently but collectively named IMPACT (Impact of non-dystrophic Myotonia on PAtients and Caregivers' qualiTy of life), were conducted to help elucidate how symptoms affect adults with NDM and those who care for adults or children with this condition. The patient survey not only confirmed NDM symptoms experienced by participants, but also highlighted how such symptoms affect a person's quality of life, mental health, and abilities including problems with work, study, childcare, and socialising. Additionally, details of the diagnostic pathway, treatment, and healthcare professionals involved in NDM were revealed. The caregiver survey found that almost one-third of those who cared for someone with NDM did so for at least 10 hours per week. It also highlighted how a carer's physical and mental health could be impacted by caregiving, potentially due to the finding that half of respondents felt that they had little or no support. Presented here are highlights of the IMPACT survey along with insights from five NDM clinical experts: Jordi Diaz-Manera, Channa Hewamadduma, Giovanni Meola, Federica Montagnese, and Sabrina Sacconi.

INTRODUCTION

Non-dystrophic myotonias (NDM) manifest relaxation as delayed muscle (myotonia) leading to muscle stiffness that diminish worsen with repeated may or contractions, depending on NDM subtype.¹ Symptoms and clinical signs, also dependent on subtype, can include transient weakness, myalgia, cramps, fatigue, dysphagia, dysphonia, and muscle hypertrophy.^{2,3}

NDM arise due to skeletal voltage-gated muscle channel defects caused by mutations in CLCN1 or SCN4A genes.3 CLCN1 codes the chloride channel CLC-1, so related conditions are classified as 'chloride channelopathies'; CLCN1 mutations lead to Thomsen myotonia congenita (TMC;4 in an autosomal dominant pattern of inheritance) or to Becker myotonia congenita (BMC;⁵ in an autosomal recessive pattern of inheritance). SCN4A mutations, also inherited in an autosomal dominant manner. code the NaV1.4 channel, with related conditions classified as 'sodium channelopathies'; these NDM include paramyotonia congenita (PMC),⁶ sodium channel myotonia, and hyperkalaemic periodic paralysis.³

Considering that NDM is a rare condition, with point prevalence estimates ranging from 0.75 to 1.70 per 100,000 people,⁷⁸ it is unlikely that many healthcare professionals (HCPs) will diagnose a patient with NDM throughout their clinical career.

IMPACT SURVEY

While descriptions NDM of symptoms found $easily,^3$ and signs can be the impact of NDM on daily life is not clearly established. A thorough analysis of this could lead to better understanding of NDM, clearer ideas about treatment, and design of scales to better monitor symptoms and treatment response.

With this in mind, between August and October 2020, market research in the form of an online survey among adults (≥18 years) with NDM and another among carers of people with NDM, collectively named IMPACT (Impact of non-dystrophic Myotonia on PAtients and Caregivers' qualiTy of life), was conducted by admedicum[®], Cologne, Germany, partnering with Lupin Neurosciences, Zug, Switzerland. The surveys were reviewed by patient experts prior to finalisation and were available in English, Dutch, French, Italian, Spanish, and German. Methods and preliminary results were presented at the 16th International Congress on Neuromuscular Diseases, 2021.9

The questioned respondents surveys on diagnosis, prior and current symptoms, abilities affected, impact on quality of life (QoL), treatment, and support. Both surveys were anonymous and confidential, with declaration of NDM/carer status completed by the respondent only. No data were collected that would permit participant identification. Respondents patient were recruited via organisations, HCPs, and social media promoted through Google advertisments.

The patient survey was completed by 181 people from 27, mostly European, countries. The highest numbers of respondents were from the USA (n=39), Germany (n=35), the UK (n=32), the Netherlands (n=15), and Spain (n=10). Respondents were predominantly female (62%) and between 31 and 65 years of age (71%), with 24% aged 18–30 years.

The caregiver survey included self-identified, non-professional caregivers of both minors (29%) and adults (71%). A total of 59 caregivers from 12 countries responded, with most from the UK (n=18), Germany (n=14), USA (n=7), Spain (n=4), Italy (n=4), and Sweden (n=4). Most respondents were female (75%), the patient's parent (42%) or partner (39%), and aged between 18 and 45 years (65%) or 46 and 65 years (32%).

At a roundtable meeting, five clinical experts, Jordi Diaz-Manera, Channa Hewamadduma, Giovanni Meola, Federica Montagnese, and Sabrina Sacconi, discussed NDM symptoms, diagnosis, treatment, and outcomes from the IMPACT surveys. Their views on the IMPACT survey results and their clinical expertise is presented here.

DIAGNOSIS

NDM diagnosis with starts ascertaining of onset, family age history, symptoms, and exacerbating factors, such as exercise, cold. menstruation, hunger, dietarv potassium, psychological stress, and alcohol.^{2,3} Key initial diagnostic steps are neurological examinations and 'bedside' tests of motor function, including observations of stiffness level when initiating movement and muscle relaxation after a contraction.³

As a consultation is only a 'spot check' of symptoms because of their unpredictability, Meola recommended patients fill out a symptom diary to help reveal exacerbating factors. This could be a paper diary or an interactive voice response system to record daily symptom severity and frequency.¹⁰

Follow-Up Tests

Electromyography can reveal electrical myotonia as distinct Fournier patterns of self-sustained bursts of discharge after a short

exercise test (sometimes with cooling).¹¹ While this can guide towards NDM categorisation, there are crossovers between the genetically distinct NDM subtypes.^{11,12}

Genetic testing can help confirm NDM subtype³ and with rapid sequencing the panel reported how wait-time for such confirmation should be only a few weeks. However, in the IMPACT survey, 19.3% of respondents had not had their diagnosis confirmed genetically.

Diagnostic Delays

The IMPACT patient survey revealed that 65% of respondents experienced symptoms for more than 10 years prior to diagnosis; for others this was 5–10 (15%) or 2–5 (12%) years. According to Hewamadduma, one reason for diagnostic delay is that younger patients may be told that symptoms are just a normal part of growing up so "they put up with [them] until maybe [...] at a sports event when the pistol goes, they can't start running."

According to Hewamadduma, once a patient presents to their general practitioner (GP), there may be a delay as "the GP may not have encountered a patient with myotonia before." Diaz-Manera agreed, saying that "sometimes [symptoms are] tightness, for others it's weakness or many other complaints that are not specific, so...for GPs it's difficult to think about congenital myotonia as an option."

Further delay may occur if a patient is not sent for appropriate specialist care. For example, instead of being referred to a neuromuscular specialist, patients might see a rheumatologist, orthopaedist, or general neurologist. While the latter may seem suitable, Diaz-Manera highlighted how even general neurologists are sometimes not familiar with NDM. This is compounded, according to Montagnese, by NDM-specific examinations not being part of a common neurological training or education. Encouragingly, Sacconi reported that in her centre, educating general neurologists to include NDM-focused electromyography has led to increased referrals of patients with NDM to neuromuscular specialists.

SYMPTOMS

Of the 181 IMPACT respondents, 28.2% had TMC, 25.4% had BMC, 17.1% had PMC, 4.4% had 'potassium-aggravating myotonia', and 2.2% had 'periodic paralysis'. The remainder reported undefined 'myotonia congenita' (11.0%), a general/other NDM diagnosis (5.0%), or were awaiting classification (6.6%).

NDM subtypes can be somewhat distinguished by age of onset and symptoms. Chloride channelopathies usually start at approximately 10 years of age.^{3,13,14} Although some BMC and TMC symptoms are similar, such as myotonia decreasing after a 'warm-up' period, others, such as muscle pain and hypertrophy, transient weakness, and upper limb involvement, are more likely in BMC, while lower limbs are predominantly affected in TMC. Sodium channelopathies tend to start at approximately 5 years of age with symptoms including facial stiffness (often involving eyelid myotonia), pain, and episodic weakness, all exacerbated in cold weather.³ In PMC especially, myotonia may increase after repetitive contractions, while for some with sodium channel myotonia, potassium consumption can aggravate the symptoms.³

Although some symptoms may differ by diagnosis, nearly all IMPACT respondents reported experiencing many of the core symptoms, such as limb muscle stiffness and mobility problems, on a daily or continuous basis and experiencing other symptoms, such as muscle pain and headache, at least sometimes (Table 1). Other symptoms reported at least sometimes were gastrointestinal issues (69%), being unable to open their eyes after sneezing/ blinking (64%), a risk of dropping drinks (62%), and their throat closing up when consuming cold drinks or food (45%).

Table 1: Symptoms experienced, quality of life factors, and emotional, social, and psychological issues listed by respondents when asked "Within the last 6 months, how often have you experienced the following symptoms?" (N=181).

	Frequency (% of respondents reporting)			
	At least sometimes*	Often/continuously ⁺		
Symptoms experienced				
Muscle stiffness/delayed muscle relaxation in legs	99%	81%		
Muscle stiffness/delayed muscle relaxation in arms	97%	73%		
Overall mobility problems	93%	68%		
Muscle stiffness/delayed muscle relaxation in face	93%	45%		
Persistent tiredness	91%	47%		
Muscle pain, aching limbs, headache, or back pain	90%	43%		
Problems taking off clothes	84%	39%		
Problems speaking	83%	20%		
Physical/muscle weakness following exercise	82%	27%		
Difficulty swallowing/chewing	80%	16%		
Inability to release grip when shaking hands	78%	33%		
Poor sleep quality	75%	33%		
Frequent falls related to stiffness	74%	10%		
Abilities affecting quality of life				
Exercising/playing sport	86%	30%		
Working/studying	73%	24%		
Socialising/communicating with others	65%	18%		
Leaving the house/carrying out basic tasks	54%	17%		

	Frequency (% of respondents reporting)				
	At least sometimes*	Often/continuously ⁺			
Carrying out simple daily tasks independently in the home	52%	16%			
Driving a car	49%	12%			
Using public transport	48%	14%			
Caring for a child	38%	11%			
Emotional, social, and psychological issues					
Lack of confidence in my abilities	82%	35%			
Constant worry due to unpredictability	80%	40%			
Worried about progression of the disease	80%	22%			
Embarrassed due to inabilities caused by symptoms	79%	32%			
Sad/depressed	78%	20%			
Social anxiety	78%	32%			
Nervous when moving in public	77%	27%			
Felt stressed from constantly comparing my inabilities to others	68%	22%			
Felt panicked/anxious	60%	17%			
Lost interest in many things they used to enjoy	65%	22%			

*Sometimes: a few times per year or month; regularly: weekly; often: usually daily; continuously: several times per day. *Often: usually daily; continuously: several times per day.

Participants could also provide free-text comments about how symptoms affected them:

- > "Crossing the street is a big problem."
- "Eating bananas, even a small piece, makes my body stiff."
- "Inability to concentrate, especially in the mornings after I have exercised the day before."
- Constant feeling of exhaustion. More than normal pain and longer restitution time needed after exercising."

QUALITY OF LIFE

NDM symptoms can greatly impact a person's QoL; while 48% of IMPACT patient respondents rated their QoL as high (at least 7 on a scale from 0 to 10 [worst to best possible condition]), 27% rated it as low (0 to 4). Many also reported constant worry due to symptom unpredictability and about NDM progression (Table 1).

IMPACT results revealed that areas of life affected included exercising/playing sport, working/ studying, and socialising/communicating with others (Table 1), as well as attending classes (36%) or finding a partner (19%). Understanding addressing factors beyond and physical symptoms could help improve the QoL for a person with NDM.¹⁵ According to Hewamadduma, the symptoms that most interfere with QoL depend on a person's specific circumstances. For example, one patient, a cricket player, had few problems practising in the summer but struggled in the winter cold. For another, their problem was being unable to walk their child to school and for a third, throat spasms when eating anything cold were problematic. Montagnese also pointed out how pain is another fundamental complaint impairing QoL.

NDM effects also include major life events. Over half (52%) of 122 participants said that NDM had restricted their abilities, indicating that it had also negatively impacted their education or career at least once. Over a quarter felt unable to apply for their preferred education/job (26%) and some had to terminate/lose a job (19%) or were not able to carry out their trained profession (17%).

Patients also expressed how NDM affected them psychologically (Table 1); for example, many felt sad or depressed, lacked confidence in their abilities, experienced social anxiety, or were embarrassed due to their limiting symptoms. Some revealed that having NDM made them feel isolated (57%), feel alone and helpless (49%), and cry a lot (49%). Over a third (39%) reported that they had been bullied due to NDM.

Respondents provided free-text comments regarding how NDM symptoms affected them psychologically/socially:

- "Fear of open spaces...severe anxiety, depression."
- "Discrimination when I go to the gym because I can't do everything...and can't tolerate bright lights and music."
- "Explaining to people who did not realise your limitations can be embarrassing."
- "Pain and fatigue have the most effect on mental state. Not having energy enough to do things others do...always having to make concessions."

To help with this aspect of NDM, Hewamadduma discussed how well-structured mental health support is essential and Meola emphasised the importance of encouraging patient networking groups, especially as there is no formal patient association for NDM.

TREATMENT

The survey results found that only 23% of respondents were 'satisfied' or 'very satisfied' with current symptom management, with 33% 'moderately satisfied' and 34% 'not satisfied.' While 29% of respondents reported they were not receiving or had never received NDM-specific treatment, 59% were currently taking/had taken a medication for NDM, 33% received physiotherapy, and 16% received psychological support.

There is currently no disease-modifying treatment for NDM; however, the Class IB antiarrhythmic mexiletine has European Medicines Agency (EMA) approval as an orphan drug to treat myotonia symptoms in adults with NDM. Mexiletine reduces or abolishes muscle hyperexcitability for both chloride and sodium ion channelopathies by inducing a slower sodium influx, thus enhancing fast inactivation and faster repolarisation of sodium channels.³ placebo-controlled Randomised, and n-of-1 clinical trials have shown mexiletine's effectiveness for reducing muscle stiffness, weakness, and pain and in improving QoL in NDM.^{10,16} Treatment requires cardiac monitoring prior to or during mexiletine as it may exacerbate arrhythmia.¹⁷ Other off-label pre-existing treatments include lamotrigine (the only other drug with a placebo-controlled trial in NDM¹⁸), carbamazepine, flecainide. acetazolamide. and ranolazine.3

Of the 89 IMPACT respondents currently taking a medication, mexiletine was the most commonly prescribed (n=45), followed by lamotrigine (n=10), flecainide (n=9), carbamazepine (n=9), acetazolamide (n=8), quinidine sulphate (n=7), calcium antagonists (n=6), phenytoin (n=2), baclofen (n=1), magnesium (n=1), or propafenone (n=1).

Figure 1 shows how often the most-experienced NDM symptoms occurred: the higher the number, the more often a symptom was experienced (1=never; 5=continuously). These were also reported as the symptoms patients most wanted improved by treatment. Also shown in Figure 1 is how much these symptoms were generally improved by drug treatment for those respondents receiving treatment: the higher the number, the more significant a symptom was improved (1=did not improve at all; 5=significant improvement).

Free-text responses regarding drug treatment included positive and negative comments. Comments were separated from other responses and aggregated so cannot be tied to a particular medication:

- "Through medication my QoL has increased.
 I can participate in more things or preserve longer."
- "In terms of my ability to communicate via eye contact, facial impressions, and gestures, my QoL has improved significantly."
- "It has definitely improved self-belief/selfconfidence that I can walk across a parking lot without getting stiff halfway across."



Figure 1: Correlation between frequency of experienced symptoms and improvement of symptoms under drug treatment for the top six symptoms experienced.

Mean for experienced symptoms: the higher the number, the more often a symptom was experienced (1=never; 5=continuously). Mean for improvement of symptoms: the higher the number, the more significantly a symptom was improved (1=did not improve at all; 5=significant improvement).

- "Overall speaking, minimal improvement of symptoms."
- "The symptoms of myotonia were hardly attenuated and other symptoms appeared that made my life quite complicated."

Patients with NDM also wanted treatment to address QoL factors and reported how their ability to carry out tasks, including leaving the house, driving, exercising, working/studying, socialising, and using public transport, was improved with drug treatment. Of note, however, the average improvement of QoL symptoms, which was also on the 1–5 scale above, was between 2–3, indicating an unmet need for more comprehensive treatment in NDM to enhance their QoL.

Targeting Treatment to Symptoms Experienced

The panel agreed that NDM treatment must consider individual needs and have a holistic understanding of each patient's behaviours, habits, and comorbidities. According to Sacconi, it is essential that the HCP and patient set treatment goals together, with Montagnese highlighting that it is vital to discuss that while the drugs are effective for some symptoms, they do not cure NDM.

Although some symptoms can be alleviated with drug treatment, Hewamadduma emphasised how in his experience, pain and tiredness are the hardest to manage: "[Patients] need a lot of counselling and goal-setting conversations, particularly those who have increased tiredness; we teach them about pacing, so [the treatment is] not only the drug itself." "Pain is something that we are not paying enough attention to," confirmed Diaz-Manera. That most patients are seeking pain relief was, he suggested, reflected in the practitioners that patients with NDM reported visiting, including an acupuncturist, kinesiologist, osteopath, and herbalist.

Addressing Those Not Receiving Drug Therapy

While drug treatment may help, 74 of the 181 NDM respondents reported they had not taken any NDM-directed medication. For some, this was simply because they did not want to take any medication (27%) or felt their symptoms were managed well enough without (16%). Indeed, according to Montagnese: "if we think the patient is not hugely affected in everyday life, if they are concerned about the drug and we ourselves are not sure they need a medication, it's right not to prescribe anything."

However, a major reason these respondents gave for not trying a drug treatment was fear of side effects (49%). "Of course, any drug has some side effects," discussed Meola, "but we need to make the right titration and spend time to educate the patient." A diary could be useful here, according to Sacconi, "to understand the best doses for therapy, [so as] not to have an adverse event."

Also reported was that some did not receive treatment because their doctor did not prescribe any (28%), or they (8%) or their doctor (9%) were not aware of NDM treatment. Indeed, the big problem, confirmed Meola, is that some neurologists do not know enough about NDM myotonia treatments to feel confident prescribing them.

Patients also, according to Sacconi, enquire about an NDM-specific diet. While no targeted approach currently exists, Meola stressed the important role of the dietitian in his practice for helping some patients to understand how myotonia could be influenced by potassium.

CAREGIVER SURVEY

While surveys of people who care for someone with other muscle-related disorders, such as muscular dystrophy, highlight how caregivers can experience considerable burden,¹⁹ to date, no such survey has been carried out regarding NDM caregivers. This was addressed by the caregivers' component of the IMPACT survey.

The diagnosis of the caregiver's charge was predominantly myotonia congenita (BMC: 37%; TMC: 12%; non-defined: 20%), followed by PMC (8%), potassium-aggravating myotonia (5%), or periodic paralysis (2%). Many reported being a caregiver for >20 years (12%), 10–20 years (37%), or 5–10 years (31%). Almost half (45%) spent \geq 5 hours per week caring, with 29% spending \geq 10 hours per week. Looking at how NDM subtype may affect care, most who cared for someone

with BMC reported spending <6 hours per week caregiving (19 of 22 caregivers), while four of seven caring for someone with TMC and four of five with PMC spent \geq 10 hours per week providing care.

Though many caregiving respondents reported a high QoL (76% rated 7 or higher on a 0–10 point, worst to best scale), some thought that their mental health (42%) and their physical health (25%) had worsened due to caregiving, with burden linked to time spent on care. Many reported that they were 'happy' to care for the person with NDM and 'felt fulfilment' from their caregiving tasks 'frequently' (69% and 36%, respectively) or 'often' (19% and 22%, respectively); however, others reported that they were 'not at all' (2% and 10%) or 'only sometimes' (3% and 19%) happy to be a caregiver or felt fulfilment from their tasks, respectively.

Additionally, many caregivers reported that they were worried about NDM progression, were concerned about the future, and were worried about their own health at least sometimes (88%, 86%, and 61%, respectively) or permanently (19%, 14%, and 0%, respectively). Carers also, at least sometimes, reported being overwhelmed by caring (61%), having inadequate sleep (54%), feeling isolated (41%), or feeling nervous (59%).

Free-text comments from caregivers were also provided:

- "I have a complete sense of helplessness as I don't know how to ease their pain or discomfort, and a sadness that they have lost out in many ways by not being able to participate in sports, drama, and physical activities."
- "All the therapies, the appointments, it takes a massive toll on the whole family not just the carer or the person affected, siblings suffer too."
- "Child has times where she is severely affected...so needs a lot of time and help with massage, getting dressed, moving up and down stairs."



Figure 2: Results from caregiver respondents when asked: "Please rate how an improvement of symptoms of the person you care for helps (or would help) to reduce the need for your care?" (multiple choices possible).

Rating scale: 1=not helpful at all; 5=significant improvement of quality of life.

Support is something that carers need as well as patients. While 51% 'regularly', 'often', or 'permanently' 'felt strongly supported by others', 27% said this was only 'sometimes' and 22% said 'not at all'. Discussing one case, Hewamadduma recounted how the mother of a child with NDM needed help because of the psychological impact of her caregiver burden. "This was not a couple of hours, this was days of psychological input as she was suicidal at one point."

A further free-text comment was provided:

"A big challenge/worry is very limited support from medical professionals who have little to no experience with NDM. As I don't know if/ how it will progress, it's worrying that they won't be able to provide me with treatment options and advice I could trust."

Caregiver burden was also reflected in the effect on carer's abilities. For example, approximately half reported that caregiving duties affected their ability to work, exercise, or pursue a hobby, meaning they had to, at least sometimes, change plans or combine caregiving with their own activities. A few reported that caregiving negatively impacted their education/career choices (six parents, one partner), that they had to reduce their working hours (five parents, one partner), or were no longer able to work (four parents, one partner).

Similar to those with NDM, caregivers reported that improvement of NDM symptoms of the person they cared for would, at least moderately, help reduce their caregiving burden (Figure 2).

CONCLUSION

The IMPACT surveys for the first-time shed light on the extent of NDM burden for people living with the disease and those caring for them. Currently, there are no formal guidelines or consensus recommendations for NDM; the IMPACT survey results could help HCPs understand unmet needs in terms of diagnosis, treatment, and support for people with NDM and their caregivers.

The results suggest that wider access to genetic testing and earlier referral to specialised neuromuscular centres is needed, which can lead to more targeted therapy according to aetiology. There are needs to develop a globally standardised NDM symptom diary; to work with patients and HCPs to create easily accessible, reliable information about NDM patient-to-patient exchanges so that they can find support and educate each other.

and treatment options; and to develop NDM Opening the survey to more people with NDM and their caregivers could add to the current findings but more data are also needed, international including co-operation and multicentric natural history studies.

References

- 1. Heatwole CR et al. The diagnosis and treatment of myotonic disorders. Muscle Nerve. 2013;47(5):632-48.
- Vereb N et al. Non-dystrophic 2 myotonias: clinical and mutation spectrum of 70 German patients. J Neurol. 2020; DOI: 10.1007/s00415-020-10328-1
- 3. Stunnenberg BC et al. Guidelines on clinical presentation and management of nondystrophic myotonias. Muscle Nerve. 2020;62(4):430-44.
- 4. Thomsen J. [Tonic cramps in voluntarily mobile muscles as a result of inherited psychological disposition (Ataxia muscularis?)]. Arch Psychiatr Nervous Dis. 1876;6(3):702-18. (In German).
- 5. Becker PE. ["On the genetics of myotonia"], Kuhn E (ed.), [Progressive Muscular Dystrophy Myotonia Myasthenia] (1966), Berlin: Springer-Verlag, pp.247-55. (In German).
- 6. Eulenburg A. [A familial form of congenital paramiotonia, which has passed down through 6 generations]. Neurol Centralbl. 1886;5:265-72. (In German).
- 7. Stunnenberg B et al. Prevalence and

mutation spectrum of skeletal muscle channelopathies in the Netherlands. Neuromuscul Disord. 2018;28(5):402-7

- 8. Horga A et al. Prevalence study of genetically defined skeletal muscle channelopathies in England. Neurology. 2013;80(16):1472-5.
- 9. Diaz-Manera J et al. Understanding the implications of non-dystrophic myotonia for patients and caregivers - results from the IMPACT survey. ePoster P742. 16th International Congress on Neuromuscular Diseases (ICNMD) 2021, Virtual meeting, 21-22, 28-29 May, 2021.
- 10. Stunnenberg BC et al. Effect of mexiletine on muscle stiffness in patients with nondystrophic myotonia evaluated using aggregated n-of-1 trials. JAMA. 2018;320(22);2344-53.
- 11. Fournier E et al. Electromyography guides toward subgroups of mutations in muscle channelopathies. Ann Neurol. 2004;56(5):650-61.
- 12. Tan SV et al. Refined exercise testing can aid DNA-based diagnosis in muscle channelopathies. Ann Neurol. 2011:69(2):328-40.
- 13. Matthews E et al. The non-dystrophic myotonias: molecular pathogenesis,

diagnosis and treatment. Brain. 2010;133(Pt 1):9-22.

- 14. Trivedi JR et al. Non-dystrophic myotonia: prospective study of objective and patient reported outcomes. Brain. 2013;136(Pt 7);2189-200.
- 15. Trip J et al. Health status in nondystrophic myotonias: close relation with pain and fatigue. J Neurol. 2009;256(6):939-47.
- 16. Statland JM et al. Mexiletine for symptoms and signs of myotonia in nondystrophic myotonia: a randomized controlled trial. JAMA. 2012;308(13):1357-65.
- 17. Suetterlin KJ et al. Mexiletine (NaMuscla) for the treatment of myotonia in non-dystrophic myotonic disorders. Expert Opin Orphan Drug. 2020;8(2-3):43-9.
- 18. Andersen G et al. The antimyotonic effect of lamotrigine in nondystrophic myotonias: a doubleblind randomized study. Brain. 2017;140(9):2295-305.
- 19. Lue YJ et al. Factors affecting the health-related quality of life of caregivers of patients with muscular dystrophy. J Neurol. 2018;265(7):1548-56.

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

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Abstract

Oxy-hydrogen gas (HHO) is a gaseous mixture of molecular hydrogen and molecular oxygen that is generated by the electrolysis of water and delivered in a 2:1 ratio (66% and 33%, respectively) through the use of noninvasive inhalation devices such as nasal cannulas or nebulisers. Although there is a paucity of scientific evidence supporting this new and emerging therapy, initial investigations indicate that HHO proffers cytoprotective qualities, typically by reducing oxidative stress and attenuating the inflammatory response. These aspects are particularly favourable when considering respiratory medicine because underlying inflammation is known to drive the pathological progress of numerous respiratory conditions, including asthma, chronic obstructive pulmonary disorder, and, pertinently, coronavirus disease (COVID-19). Direct delivery to the lung parenchyma is also likely to increase the effectiveness of this emerging medical therapy.

This narrative review aims to delineate how this particular combination of gases can affect cellular processes at the molecular level by focussing on the evolutionary requirement for both oxygen and hydrogen. Furthermore, the authors assess the current available data for the safety and efficacy of HHO in a clinical setting.

INTRODUCTION

During both acute and chronic respiratory conditions, including severe coronavirus disease (COVID-19) infection, the primary physiological response is to synthesise distress proteins, which include cytokines, chemokines, and growth factors. This phenomenon is known as the cytokine storm, an event which can exacerbate the inflammatory response and rapidly increase the host's temperature in an attempt to eradicate pathogens.¹ Such proinflammatory mediators increase the production of reactive oxygen species (ROS) both within the mitochondrial electron transport chain (ETC) and within recruited immune cells, such as neutrophils and macrophages,² by the activation of enzymes such as NADPH oxidases. Mitochondrial dysfunction often occurs when cellular demand for the high-energy phosphate molecule ATP increases during times of cellular stress.³ This situation can lead to congestion in the flow of electrons within the ETC, resulting in excessive leakage of electrons. Rogue electrons are free to combine with molecular oxygen at will, forming free radicals including the superoxide anion, which dismutates to hydrogen peroxide. The reaction within the immune response cells, however, is entirely different. The mitochondrial content within these cells is considerably lower than in somatic cells, and therefore is not a significant source of ROS. Instead, granulocytes utilise NADPH oxidase enzymes to produce a highly oxidative respiratory burst containing a myriad of ROS,⁴ which can include superoxide anions, hydrogen peroxide, hydroxyl radicals (in the presence of iron [Fe] ions), and hypochlorous acid, the latter through the action of myeloperoxidase. Mounting evidence suggests this is important when considering respiratory medicine because elevated levels of granulocytic cells are often observed in the bronchoalveolar lavage fluid of patients with astringent pulmonary diseases.^{5,6}

ROS delivered by such granulocytes can be extremely damaging to biomolecules, including proteins, carbohydrates, lipids, and nucleotides, with intense and acute elevation of ROS consistent with acute and chronic inflammatory conditions.⁷ Within the delicate parenchymal tissue, such assault often induces degeneration of epithelial cells, producing hyalin fluid. This process is known to contribute significantly to cellular hyperplasia and reduce the capacity for oxygen exchange. Raised ROS levels can also directly modify DNA nucleotides, affecting genetic transcription, and form adducts upon essential epigenetic and metabolic enzymes.⁸⁻¹⁰ Such post-translational oxidative modifications to either nucleotides or proteinaceous enzymes often lead to aberrant enzymatic activity, fluctuation causing of essential energyproducing processes and affecting mitochondrial as influencing function, well as genetic transcription events.

HHO is an emerging medical gas, generated from the electrolysis of water. HHO inhalation is a noninvasive therapy done via a nasal cannula or mask, allowing the gas mixture to directly enter the respiratory system. In addition to oxygen delivery, HHO also provides molecular hydrogen (H_2) promptly to the lung parenchyma, the target tissue for respiratory infections. Research into H_2 therapy is rapidly gathering momentum and studies have identified H_2 as having antioxidant scavenging properties,^{11,12} neutralising the deleterious hydroxyl radical and peroxynitrite ion. Presumably, other modes of action of H_2 are also important as the reaction kinetics between H_2 and these respective biomolecules remain in question.¹³

Despite research into the efficacy of HHO therapy still being in its infancy, HHO inhalation has been recommended by the Chinese government as a treatment of nosocomial COVID-19 symptomology.¹⁴

AIMS

As the global COVID-19 pandemic threatens both individual health and healthcare systems globally, and, as yet, there is little effective treatment for this novel disease, this review aims to explain the HHO generation process and the molecular benefits of combining oxygen and hydrogen inhalation therapy for COVID-19 and other inflammatory respiratory conditions. The authors have collated data from empirical and evolutionary studies and in-human clinical trials and offer an explanation as to how the molecular mechanisms behind HHO therapy may be beneficial for respiratory health.

DISCUSSION

Generation, Delivery, and Safety Profile of Oxy-hydrogen

HHO is a stoichiometric mixture of H_2 (66%) and O_2 (33%) obtained through the electrolysis of water (H_2O). As pure water has a low electrical conductivity and requires an excess of energy to degrade the hydrogen bonds that hold the molecule together, it is therefore necessary to add a water-soluble electrocatalyst (e.g., K⁺, Mg²⁺, or Na⁺) to reduce the activation threshold.¹⁵ HHO gas can be easily generated by adding electrolyte compounds, such as sodium or potassium hydroxides, to distilled water and applying a low-voltage (9-13 V) direct current.¹⁶ The O_2 and H_2 would collect in the gas phase as the water would become rapidly saturated and gases such

as H_2 have a relatively low solubility, as discussed by Hancock et al.¹⁷

In its simplest form, an electrolyser cell contains two electrodes, the positive cathode and a negative anode, separated by an ion exchange membrane. Passing an electrical current through the water decomposes H_2O into H^+ and $OH^$ ions. These electrophilically charged molecules are then attracted to the opposingly charged electrode where they liberate as gases. The chemical activity at the anode is $2H_2O \rightarrow O_2 + 4H^+$ + 4e⁻ whilst the activity at the cathode equates to $4H^+ + 4e^- \rightarrow 2H_2$. This method ensures separation utilising the $2H_2O \rightarrow 2H_2 + O_2$ reaction without returning it to the aqueous state.

As interest grows in this particular field of research, the industry surrounding the development of HHO products is also expanding. An HHO generator's standard specification incorporates an electrolysis unit, a filter, and an electronic control unit. The control unit can adjust the voltage directed to the positive and negative plates. Once formed, HHO can enter the respiratory system directly by inhalation through either a nasal cannula or an inhalation mask at a peak rate of 2–3 L/min. Gas inhalation through nasal cannulas is a safe and noninvasive delivery method widely used globally for pure oxygen inhalation within healthcare settings.

Although being comprised of the highly flammable gases hydrogen and oxygen, known to reduce the combustion threshold for most substances, the safety profile of HHO in a clinical setting can be regarded as favourable. This is partly because of the high autoignition threshold of HHO (570 °C),18 the contained generation within a sealed system, and because HHO typically gets used as generated. Although the likelihood of explosion when using an HHOgeneration device is negligible, consideration over the combustion potential in the presence of a naked flame is warranted; consequently, activities that involve use of a flame, including smoking, during treatment pose a significant risk of igniting HHO and causing potential harm.

The Requirement for Oxygen

Rapid oxygenation of the Earth's atmosphere, known as the Great Oxygen Event, took place approximately 2 billion years ago during the early Paleoproterozoic era.¹⁹ It is assumed that this event was triggered by photosynthesising cyanobacteria that produced oxygen as a waste product.²⁰ As the rapid rise in atmospheric O₂ presented a new challenge for cellular energetics, single-celled life forms were forced to adapt, including giving rise to multicellular life.

On a cellular level, O₂ is integral to the process of energy generation during aerobic respiration. The cardinal role here is for oxygen to act as a terminal electron acceptor during aerobic respiration.²¹ Aerobic respiration is the biochemical process of ATP synthesis that occurs in the mitochondria. ATP synthesis is crucial to all cellular processes as it is a high-energy molecule responsible for donating energetic phosphate moieties during cellular metabolic pathways.

With eight electrons and eight protons in each molecule, O2 is an electrophilically neutral diradical compound with two unpaired electrons in its outer shell. This neutrality allows the O₂ molecule to easily diffuse through cellular membranes and into the mitochondria where it is required to accept electrons donated by complex IV (cytochrome oxidase) in the ETC. If oxygen is not present to fulfil this role, aerobic respiration, responsible for producing a theoretical 38 ATP per glucose molecule, discontinues and the less efficient anaerobic respiratory pathway is initiated, generating a mere two ATP per glucose molecule.²² Prolonged anaerobic activity is unsustainable in higher life forms as the energy produced is insufficient to sustain essential cellular functions. Granulocytic leukocyte cells, such as neutrophils, rely much more on glycolytic production of ATP because of a lack of mitochondrial presence in these specific cells,^{23,24} a factor that allows these cells to continue functioning even during hypoxaemia where oxygen supply is notably reduced.

As described above, oxygen is fundamental to the survival of higher organisms and the average adult (63 kg) consumes approximately 200 mL of O_2 every minute at standard temperature and pressure,²⁵ equating to 400 L per day. Once O_2 is inhaled into the lungs from the atmosphere, it is exchanged for CO_2 as O_2 has a higher binding affinity with iron-containing haemoglobin in red blood cells. Healthy saturation of haemoglobin, a measurement of the percentage of haemoglobin molecules that carry O_2 , is between 94% and 98%.²⁶ Saturation levels below this threshold typically lead to hypoxaemia, which if unrectified can lead to hypoxia: a condition where tissues do not receive adequate amounts of O_2 . This situation can progress to acute respiratory distress syndrome, multiple organ failure, and potentially death if left untreated.

Estimated oxygen usage in ambulances in the UK is reported to be in excess of 2 million times each year, equating to 34% of attended call-outs.^{26,27} In these cases, O₂ therapy is not typically used to alleviate the symptoms of respiratory distress; instead, it is often used to prevent hypoxaemia in emergency conditions, including cardiac arrest and trauma. Oxygen is given to approximately one in five patients in nosocomial care.²⁷ In this setting, O₂ inhalation is prescribed for a vast array of life-threatening conditions, including those that cause a rapid onset of depressed breathing, such as anaphylaxis, COVID-19, opioid overdose, and pneumonia, as well as for persistent hypoxaemic complaints. By providing O, therapy, clinicians are able to increase oxygen tension in the alveoli during such events, thereby decreasing the respiratory system's workload by fulfilling the cellular demand for O2.28 Although widely used in emergency medicine, it is important to note that oxygen is also prescribed for the alleviation of chronic respiratory complaints such as asthma, chronic obstructive pulmonary disorder (COPD), cystic fibrosis, and paediatric respiratory disease.

The Requirement for Molecular Hydrogen

As the most basic element within the universe, it may come as no surprise that molecular hydrogen has played a pivotal role in the evolutionary process. One intriguing theory, highlighted by Andersson and Kurland,²⁹ implies that the bacterial endosymbiont's original function was to provide the host cell with H_2 .

The hydrogen hypothesis advocates for an archaeon host cell with a facultative anaerobic means of respiration, making use of CO_2 as a metabolic substrate and producing H_2 as a by-product, instead of being a committed aerobe with an immediate demand for ATP as suggested in the traditional endosymbiotic theory.²⁹ Indeed, the last universal common ancestor has been described as having all enzymes associated with both mitochondria responsible

for ATP production in aerobic organisms, and hydrogenosomes that facilitate metabolic processes in anaerobic organisms. Their later divergence can be explained by environmental pressures that ultimately led to natural selection events.

Although this debate has yet to be rectified, it has raised some thought-provoking questions worthy of further exploration. When considering the hydrogen hypothesis, of particular appeal is the homologous protein expression profile noted within both mitochondria and hydrogenosomes.³⁰ Despite both organelles differing in their biochemical activities, they each have the capacity to encode for proteins of both complex I and II of the ETC. However, it is only the mitochondrial genome that can encode for elements of complexes III-IV and, importantly, ATP synthase. If, as suggested by Lewis et al.³¹ and others,^{32,33} hydrogenosomes and mitochondria are delineated from the same original symbiont, it is likely that mitochondria once had the ability to produce H_{a} .

Yet another link to the evolutionary requirement for H₂ in eukaryotes are the hydrogenase enzymes found in many species of photosynthesising organisms, including algae and higher plants. To exemplify, the single-celled green alga Chlamydomonas reinhardtii contains two Fe- hydrogenases (HYDA1 and HYDA2) that can receive electrons from ferredoxin during photosynthesis,³⁴ reducing proton accumulation with the reaction $2H^+ + 2e^- \rightarrow H_2$. Furthermore, homologous hydrogenase enzymes are also found in higher plants, including crops such as the legume Medicago truncatula³⁵ and Oryza sativa subsp. japonica (rice).³⁶ The frequency and diversity with which hydrogen-producing mechanisms have occurred along the phylogenic tree suggest an inherent biological requirement for H₂ across all domains of life.

In addition to the activity of hydrogen-producing enzymes and organelles in eukaryotic species, many anaerobic bacteria metabolise H₂. Typically, bacterial generation of hydrogen is linked with transition metal-containing (e.g., nickle/iron [NiFe] or iron/iron [FeFe]) hydrogenase enzymes that catalyse the reversible oxidation of hydrogen with the reaction $2H^+ + 2e^- \rightarrow 2H_2$.³⁷ Interestingly, hydrogen-producing bacteria are known to form symbiotic relationships with both plants and animal species, which may also fulfil the cellular requirement for H_2 in multicellular organisms, including humans. Here, H_2 is largely obtained from the symbiotic relationship between H_2 -producing intestinal microflora (e.g., *Escherichia coli* or *Clostridium butyricum*) and the host.³⁸

Aside from the largely theoretical requirement for H_a in the early development of life, contemporary research is beginning to reveal that molecular hydrogen can act as a natural antioxidant in at least two possible ways: 1) through the selective scavenging of nonsignalling ROS/reactive nitrogen species, such as the hydroxyl radical and peroxynitrite, thus potentially reducing oxidative damage to cellular membranes and the inactivation of metabolic enzymes through oxidative post-translational modifications.³⁹ It should be made clear that the reaction kinetics here are unfavourable¹³ and more research is needed before these claims can be verified and universally accepted; and, 2) via increased activity of nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor responsible for upregulating the activity of the antioxidant response element, a cis-acting enhancer sequence located within the promotor region of more than 200 genes known to transcribe and translate antioxidant enzymes (e.g., catalase and superoxide dismutase) and cytoprotective proteins and peptides (e.g., glutathione-Stransferase and cytochrome P450 isoenzymes).⁴⁰

In addition to the antioxidant effect H₂ clearly provides, there is also a wealth of laboratory evidence that describes H₂ as having both immunomodulatory and antiapoptotic effects in various plant and animal species. This may be largely attributed to increased activity of Nrf2.41 To illustrate, increased Nrf2 activity has been demonstrated to causally undermine expression profiles of NF-kB,42 a transcription factor associated with innate immunity and one responsible for promoting genetic transcription of numerous proinflammatory molecules.43 As well as downregulating the proinflammatory response at the genetic level, Nrf2 is known to respond to and upregulate expression of haem oxygenase, an enzyme that has a canonical function in reducing and preventing vascular inflammation.⁴⁴ Also of interest, particularly when considering the broader immune response, is the influence of NF-kB on adaptive immune cells. Here, NF-kB can provoke and stimulate

inflammatory T-cell differentiation.^{45,46} Therefore, enhancing or even prolonging the activity of Nrf2 within the nucleus is likely to have a significant effect on the inflammatory profile during disease.

The Benefits of Oxy-hydrogen in Respiratory Medicine

Although, as previously stated, research into the benefits of HHO as a medical gas is at an early stage, the recent pandemic concerning the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has highlighted the need for emergency respiratory care that is effective in reducing parenchymal inflammation. This need becomes of particular relevance as inflammatory exudate is known to contribute to the impairment of the alveoli's oxygen-exchange capacity, further exacerbating hypoxaemic conditions during severe infection. In a recent study involving murine models of asthma conducted by Zhang et al.,47 1 hour of HHO inhalation per day was demonstrated to decrease airway resistance and reduce epithelial cell hyperplasia. Furthermore, significant reductions in the expression of proinflammatory mediators were identified, including chemokines (e.g., CXCL15), cytokines (e.g., TNF- α), and multiple interleukins (e.g., IL-4 and IL-6).47 These findings may prove to be particularly relevant as tocilizumab, an IL-6 intervention therapy, is currently being used to treat COVID-19 as well as autoimmune and other severe inflammatory conditions.48 However, the benefits of this treatment may be outweighed by the possible side effects, which include an increased occurrence of upper respiratory tract infections, hypertension, and gastrointestinal perforation.49

Despite the growing popularity of HHO as a potential medical therapeutic, there is, as yet, sparse evidence on the clinical safety of using HHO generators; however, initial in-human trials into the effects of HHO in patients with tracheal stenosis reported no adverse effects of treatment (n=35).⁵⁰ Additionally, trials into the effects of HHO supplementation during severe COVID-19 infection are ongoing in the Hubei province of the People's Republic of China,⁵¹ with Chinese health officials recommending HHO therapy in the national treatment protocol for coronavirus-associated pneumonia.¹⁴

Additionally, during a 3-month, in-human trial assessing the safety and efficacy of HHO inhalation therapy, a curative effect was noticed from the second week until the end of the trial in patients (n=70) with severe COPD.52 These findings are further supported by results from a 10-day, multi-centre, randomised, doubleblind study that reported easement of COPD symptoms on observing days 1-7 (n=54).53 Similar findings have also been reported in studies of chronic inflammatory respiratory diseases, including asthma, cystic fibrosis,⁵⁴ and COVID-19.55 Here, increased oxygenation and the cellular protective effects of molecular hydrogen has been shown to recover poor blood saturation levels of O2 and reduce the inflammatory thus preventing and rectifying response, hypoxaemia and reducing the likelihood of Type 1 respiratory failure.⁵⁷

Future Perspectives

Research into the biochemical properties of medical gases is beginning to reveal that, alongside O₂, H₂ may also be of evolutionary significance, perhaps by acting as part of the suite of gaseous signalling molecules. However, laboratory trials utilising human-derived cell cultures and animal models will be necessary if the molecular mechanisms that influence somatic responses are to be elucidated. *In silico* models of distribution patterns, reaction kinetics, and longevity will also be required if a full understanding of signalling behaviours is to be reached. Furthermore, as much of the clinical data available have yet to be substantiated,

data obtained from patient trials will need to be hastened if HHO therapy is to be widely used as a treatment in general medicine.

CONCLUSION

HHO treatments become of particular relevance when considering the increasing occurrence of highly contagious and novel viruses; therefore, there is an urgent need for sustainable and effective treatment protocols that can alleviate the most severe symptoms of such respiratory tract infections without the need for critical care.

Emerging clinical and preclinical^{54,55} data suggest that HHO inhalation is both safe and efficacious in treating inflammatory-related pulmonary disorders. Interestingly, despite objective research into the efficacy of HHO therapy being relatively new, initial findings suggest inhalation of these gases in stoichiometric proportions can provide symptomatic relief from chronic and acute respiratory conditions, with asthma, COPD, and cystic fibrosis being examples. Therefore, it is conceivable that combining oxygen, a substance known to alleviate respiratory distress by increasing alveolar oxygen tension and improving O₂ saturation of haemoglobin, with hydrogen, an element that displays anti-inflammatory and antioxidant qualities, in analeptic doses reduces the significant burden on lung parenchyma during disease. In order to confirm the data generated by preliminary investigations into HHO inhalation, further clinical, preclinical, and laboratory analysis is necessary.

References

- Guo C et al. Single-cell analysis of two severe COVID-19 patients reveals a monocyte-associated and tocilizumab-responding cytokine storm. Nat Comm. 2020;11(1):3924.
- Zinovkin RA et al. Role of mitochondrial reactive oxygen species in age-related inflammatory activation of endothelium. Aging. 2014;6(8):661-74.
- Brand MD, Nicholls DG. Assessing mitochondrial dysfunction in cells. Biochem J. 2011;435(2):297-312.
- Rastogi R et al. NOX activation by subunit interaction and underlying mechanisms in disease. Frontiers Cell Neurosci. 2017;10:301.

- Goldstein RA et al. Clinical role of bronchoalveolar lavage in adults with pulmonary disease. Am Rev Respir Dis. 1990;142(2):481-6.
- Costabel U et al. 2019. Bronchoalveolar lavage. ERS Handbook of Resp. Med. 2019;138.
- Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. Am J Physiol Lung Cell Mol Physiol. 2002;283(2):L246-55.
- Heiss H et al. Regulation of eNOS enzyme activity by posttranslational modification. Curr Pharm Des. 2014;20(22):3503-13.
- 9. Whitaker AM et al. Base excision repair of oxidative DNA damage:

from mechanism to disease. Front Biosci. 2017;22:1493-522.

- Russell G et al. Is glyceraldehyde-3phosphate dehydrogenase a central redox mediator? React Oxyg Species. 2020;9(26):48-69.
- Ohsawa I et al. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. Nat Med. 2007;13(6):688-94.
- Ishibashi T et al. Therapeutic efficacy of molecular hydrogen: a new mechanistic insight. Curr Pharm Des. 2019;25(9):946-55.
- Penders J et al. ONOOH does not react with H₂: potential beneficial effects of H₂ as an antioxidant by

selective reaction with hydroxyl radicals and peroxynitrite. Free Radic Biol Med. 2014;75:191-4.

- National Health Commission (NHC) of the PRC, National Administration of Traditional Chinese Medicine of the PRC. Guidance for corona virus disease 2019: prevention, control, diagnosis and management. 2020. Available at: http://www.pmph.com. Last accessed: 25 January 2021.
- 15. Streblau M et al. The influence of the electrolyte parameters on the efficiency of the oxyhydrogen (HHO) generator. 18th Int Symp on Electrical Apparatus and Tech. 2014;2014:1-4.
- Essuman SPK et al. Experimental studies of the effect of electrolyte strength, voltage and time on the production of brown's (HHO) gas using oxyhydrogen generator. Open J Energy Efficiency. 2019;8(2):64-80.
- Hancock JT et al. Downstream signaling from molecular hydrogen. Plants. 2021;10(2):367.
- Kumar GA, Rao GV. Performance characteristics of oxy hydrogen gas on two stroke petrol engine. Int J of Engineering Trends and Tech. 2013;6(7):358-66.
- Gumsley AP et al. Timing and tempo of the great oxidation event. Proc Natl Acad Sci. 2017;114(8):1811-6.
- 20. Sánchez-Baracaldo P, Cardona T. On the origin of oxygenic photosynthesis and cyanobacteria. New Phytologist. 2020;225(4):1440-6.
- Dunn JO et al. Physiology of oxygen transport. BJA Education. 2016;16(10):341-8.
- 22. Hagman A, Piškur, JA. Study on the fundamental mechanism and the evolutionary driving forces behind aerobic fermentation in yeast. PLoS One. 2015;10(1):e0116942.
- 23. Fels AO, Cohn, ZA. The alveolar macrophage. J Appl Physiol. 1986;60(2):353-69.
- 24. Jeon JH et al. Current understanding on the metabolism of neutrophils. Immune Net. 2020;20(6):e46.
- 25. Loer SA et al. How much oxygen does the human lung consume? Anaesthesiology. 1997;86(3):532-7.
- National Institute for Health and Care Excellence (NICE). Clinical guide for the optimal use of oxygen therapy during the coronavirus pandemic.
 2021. Available at: https://www.nice. org.uk/treatment-summary/oxygen. html. Last accessed: 31 January 2021.
- 27. Dhruve H et al. Oxygen therapy: emergency use and long-term

treatment. Lung cancer. 2015;15:(05).

- O'Driscoll BR et al. BTS guideline for emergency oxygen use in adult patients. Erratum in: Thorax. 2008;63(6):1-68.
- Andersson SG, Kurland CG. Origins of mitochondria and hydrogenosomes. Curr Opin Microbiol. 1999;2(5):535-41.
- Bui ET et al. A common evolutionary origin for mitochondria and hydrogenosomes. Proc Natl Acad Sci. 1996;93(18):9651-6.
- Lewis WH et al. Convergent evolution of hydrogenosomes from mitochondria by gene transfer and loss. Mol Biol Evol. 2020;37(2):524-39.
- Embley TM et al. Hydrogenosomes, mitochondria and early eukaryotic evolution. IUBMB Life. 2003;55(7):387-95.
- Martin W et al. An overview of endosymbiotic models for the origins of eukaryotes, their ATPproducing organelles (mitochondria and hydrogenosomes) and their heterotrophic lifestyle. Biol Chem. 2001;382(11):1521-39.
- Russell G et al. Hydrogenases and the role of molecular hydrogen in plants. Plants. 2020;9(9):1136.
- Scholte M et al. T-DNA tagging in the model legume Medicago truncatula allows efficient gene discovery. Molecular Breeding. 2002;10(4):203-15.
- Kesawat MS et al. Genome-wide identification, evolutionary and expression analyses of putative Fe-S biogenesis genes in rice (Oryza sativa). Genome. 2012;55(8):571-83.
- Fontecilla-Camps JC et al. Structure/ function relationships of [NiFe]-and [FeFe]-hydrogenases. Chem Rev. 2007;107(10):4273-303.
- Nandi R, Sengupta S. Microbial production of hydrogen: an overview. Crit Rev Microbiol. 1998;24(1):61-84.
- Jeffery CJ. Protein moonlighting: what is it, and why is it important? Philos Trans R Soc Lond B Biol Sci. 2018;373(1738):20160523.
- 40. Raghunath A et al. Antioxidant response elements: discovery, classes, regulation and potential applications. Redox Biol. 2018;17:297-314.
- He F et al. Nrf2, a transcription factor for stress response and beyond. Int J Mol Sci. 2020;21(13):4777.
- Ahmed SMU et al. Nrf2 signaling pathway: pivotal roles in inflammation. Biochimica et Biophysica Acta. 2017;1863(2):585-97.

- Liu Q et al. A protective role of the NRF2-Keap1 pathway in maintaining intestinal barrier function. Oxid Med Cell Longev. 2019;2019:1759149.
- 44. Araujo JA et al. 2012. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. Front Pharmacol. 2012;3:119.
- 45. Yamamoto Y, Gaynor RB. Role of the NF-kB pathway in the pathogenesis of human disease states. Current Mol Med. 2001;1(3):287-96.
- Suzuki T et al. Systemic activation of NRF2 alleviates lethal autoimmune inflam-mation in scurfy mice. Mol Cell Biol. 2017;37(15):e00063-17.
- 47. Zhang N et al. Inhalation of hydrogen gas attenuates airway inflammation and oxidative stress in allergic asthmatic mice. Asthma Res Pract. 2018;4(1):3.
- Fu B et al. Why tocilizumab could be an effective treatment for severe COVID-19. J Transl Med. 2020;18(1):164.
- 49. Heinzerling L et al. Checkpoint Inhibitors: the diagnosis and treatment of side effects. Dtsch Arztebl Int. 2019;116(8):119-26.
- 50. Zhou ZQ et al. Breathing hydrogenoxygen mixture decreases inspiratory effort in patients with tracheal stenosis. Respiration. 2019;97(1):42-51.
- 51. Guangzhou Institute of Respiratory Disease. Hydrogen/oxygen mixed gas inhalation for coronavirus disease 2019 (COVID-19). NCT04378712. http://clinicaltrials.gov/show/ NCT04378712.
- 52. Shanghai Asclepius Meditec Inc. Adjuvant therapy for severe COPD patients in the stable Phase by an oxyhydrogen generator with nebulizer. NCT02850185. http:// clinicaltrials.gov/show/NCT02850185.
- 53. Shanghai Asclepius Meditec Inc. A therapy for improving symptoms in patients with acute exacerbations of COPD by hydrogen-oxygen generator with nebulizer. NCT04000451. http:// clinicaltrials.gov/show/NCT04000451.
- 54. McCarthy SD et al. Future trends in nebulized therapies for pulmonary disease. J Pers Med. 2020;10(2):37.
- 55. Guan W et al. Hydrogen/oxygen mixed gas inhalation improves disease severity and dyspnea in patients with coronavirus disease 2019 in a recent multi-centre, openlabel clinical trial. J Thorac Dis. 2020;12(6):3448-52.
- 56. Shebl E, Burns B. Respiratory Failure. StatPearls. 2020;30252383.

Prevalence of Scoliosis in Hypermobile Ehlers-Danlos Syndrome

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Abstract

Objective: The main purpose of this study was to evaluate the prevalence, form, and severity of scoliosis in a population of adults meeting the 2017 criteria for hypermobile Ehlers-Danlos syndrome (hEDS). The second objective was to compare the prevalence of scoliosis versus other criteria at initial hEDS diagnosis.

Methods: A retrospective study looking at the frequency and severity of scoliosis in adults (N=28) meeting the 2017 diagnostic criteria for hEDS through analysis of a full spine EOS[®] X-ray (EOS imaging, Paris, France) performed at the initial diagnosis. Severity was defined by the Cobb angle.

Results: At the initial diagnosis, the mean age was 30.1 years (standard deviation [SD]: \pm 10.18 years). Twenty-nine percent (n=8/28) of patients fulfilling hEDS criteria presented with scoliosis. Thirty-two percent (n=9/28) of patients had scoliotic inflection and 39% (n=11/28) had no scoliosis. Scoliosis was mild-to-moderate with a mean Cobb angle of 13.6° (SD: \pm 3.5°). None of the patients had severe scoliosis requiring surgery. Compared to the 2017 diagnostic criteria, it is noteworthy that scoliosis prevalence in this present study population ranks at the level of the most frequent ones.

Conclusion: This study provides interesting information regarding frequency of scoliosis and scoliotic inflection in a group of patients with hEDS. Although the patients did not present with severe forms of scoliosis at initial diagnosis, the results highlight the importance of systematically looking for scoliosis in adult and young patients, in order to follow progression and ensure appropriate management.

INTRODUCTION

Joint hypermobility is often asymptomatic.¹ It can sometimes progress to joint instability, causing sprains, dislocations, or pain suggestive of Ehlers-Danlos syndrome (EDS). Joint hypermobility can also be associated with other pathologies, such as neuromuscular diseases, Marfanoid syndromes, or skeletal dysplasia.¹ The diagnostic process for symptomatic joint hypermobility must be rigorous and structured to guide the diagnosis.

EDS are a heterogeneous group of inherited connective tissue disorders caused by different mutations in genes involved in the structure or biosynthesis of collagens and extracellular matrix proteins.² The collagens involved are Types I, III, V, and XII.² Some EDS are the result of abnormalities in the synthesis of glycosaminoglycans.³ There are currently 14 types of EDS.^{2,4} The phenotypic hallmarks include joint hypermobility, skin hyperextensibility, and tissue fragility. The prognosis and management of each type of EDS are different, requiring precise clinical diagnosis and genetic confirmation. EDS are rare diseases, with an estimated global frequency of one in 5,000.⁵ Inheritance is most often autosomal dominant but with variable expression in the same family.³ Hypermobile EDS (hEDS) is the most common type.6 It mainly affects women.7 Its diagnosis remains clinical, in the absence of clearly identified molecular bases. An interview on personal and family clinical history, followed by a rigorous clinical examination using a checklist of diagnosis criteria, confirms the diagnosis.² It is cautious and useful, especially with young patients and adolescents, to reassess the patient several times before confirming the diagnosis of hEDS.

Scoliosis is a structural deformity of the spine in the three dimensions of space. The diagnosis is confirmed when the Cobb angle is 10° or higher and axial rotation is recognised on the X-ray. It should not be confused with a scoliosis attitude, which originates outside the spine (such as in length inequality of the lower limbs or pelvic rotation) and is totally reducible when patients lie down. It normally does not progress to scoliosis.⁸

Twenty percent of scoliosis are not idiopathic and may be linked to an underlying disease with hypermobility; as a consequence, it was one of the clinical criteria discussed but not included during the establishment of the new 2017 criteria for hEDS.⁹

Limited data exist in the literature regarding the prevalence and severity of scoliosis in EDS and, in particular, in hEDS.¹⁰⁻¹² The main objective of this study was to assess the prevalence, form, and severity of scoliosis in hEDS through a retrospective study in a homogeneous population of adults fulfilling the 2017 criteria for hEDS. The second objective was to compare the prevalence of scoliosis relative to the prevalence of other clinical features at diagnosis.

METHODS

Patient Selection

This was a retrospective study based on the analysis of medical records of patients who visited the reference centre of rare diseases between 2017 and 2020. Twenty-eight patients were selected. Eligibility criteria included patients aged \geq 18 years, fulfilling the 2017 criteria for hEDS, and who had a full spine X-ray at the time of diagnosis. ^{2,13} Pregnant patients and those without full spine X-ray available were not included in the study.

Diagnostic Procedures for hEDS and Evaluation of Generalised Joint Hypermobility

Each patient had been clinically examined by an expert physician from the reference centre of rare diseases to confirm hEDS diagnosis. The 2017 diagnostic criteria were evaluated to confirm the diagnosis, following the checklist provided by the international EDS consortium.¹³

During the examination, patients underwent a generalised joint hypermobility measure using the Beighton score.^{1,14,15} Each hypermobile joint scores one point, making it possible to define a total score out of 9. The positivity threshold must be adapted according to age.¹⁶ Authors consider generalised joint hypermobility with a score of 5 out of 9 for men and women <50 years old, and 4 out of 9 in adults over >50 years old; in prepubertal children and adolescents, the threshold is 6 out of 9 (Box 1A). The interobserver reproducibility is rather good (kappa: 0.80).¹⁷ Because joint laxity decreases with age in the

general population and in hypermobile patients, the Hakim and Grahame simplified questionnaire (five-part questionnaire) was also used to evaluate history of hypermobility.¹ Positive response to two or more questions between five items brings an additional point to the measured Beighton score (Box 1B). The Beighton score and the Hakim and Grahame simplified questionnaire are two criteria of the 2017 criteria for hEDS.

An objective of clinical examination was also to eliminate other connective tissue disorders (e.g., other types of EDS, Marfan syndrome, and Loeys-Dietz syndrome). Moreover, according to 2017 recommendations, all the patients underwent a cardiac ultrasound to evaluate both the mitral valve and aortic root.²

Detection of Scoliosis Using Full Spine X-ray

During the measure of the Beighton score, the authors evaluated the ability for patients

to put their hands flat on the floor with knees extended; this evaluation would unmask the presence of thoracic or lumbar scoliosis. After checking for pregnancy, patients with hEDS benefited from a complete spine radiographic examination using the EOS® method (EOS imaging, Paris, France). The EOS methodology uses a radiation dose 10-times lower than conventional radiology techniques, 1,000-times lower than that of a scanner, and optimises the follow-up and management of patients with osteoarticular pathologies of the spine and lower limbs (e.g., scoliosis and static disorders). The reconstructions in two- or three-dimensions, with lateral, frontal, and horizontal views, allow a very precise analysis of the deformities of the spine, from the head to the pelvis. This system is also of interest in terms of therapeutic monitoring.

Box 1: Beighton score and five-part questionnaire for generalised joint hypermobility.

Α	Dorsiflexion of the 5 th metacarpophalangeal joint to >90°	1 point for right, 1 point for left		
	Opposition of the thumb to the volar aspect of the forearm	1 point for right, 1 point for left		
	Hyperextension of the elbows to >10°	1 point for right, 1 point for left		
	Hyperextension of the knees to >10°	1 point for right, 1 point for left		
	Ability to place hands flat on the floor with knees fully extended	1 point		
	Total score	/9		

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Can you now or could you ever touch your hands flat on the floor without bending your knees?	Yes	No
Can you now or could you ever bend your thumb to touch your forearm?	Yes	No
As a child, did you amuse your friends by contort-ing your body into strange shapes or could you do the splits?	Yes	No
As a child or teenager, did your shoulder or knee-cap dislocate on more than one occasion?	Yes	No
Do you consider yourself 'double jointed'?	Yes	No

A) Beighton score for generalised joint hypermobility measure. A positive Beighton score is \geq 5/9 for adults; \geq 4/9 for adults over the age of 50 years; and \geq 6/9 points for children and adolescents. **B)** Five-part questionnaire for generalised joint hypermobility. A positive response to two or more questions brings one additional point to the Beighton score.

The EOS report included the measurement of sub-pelvic parameters (pelvic incidence, sacral slope, and pelvic version), the existence of disorders of the frontal or sagittal balance of the spine, the existence of a scoliotic inflection, scoliosis, its type (thoracic, thoracolumbar, or lumbar), and the Cobb angle measured for each curvature. Other spinal abnormalities such as spondylolisthesis, history of Scheuermann's disease, and vertebral abnormalities could also be detected. A classification of morphotypes defined by Roussouly in 2005 allows different types of sagittal profiles to be identified.¹⁸ Of interest in this classification (which has only been validated in adults) is its ability to understand degenerative disorders of the spine. Morphotypes with low pelvic incidence (Type 1 and 2) will be more prone to degenerative pathologies of the disc and pain, while morphotypes with high pelvic incidence (Type 3 and 4) will be more at-risk of degenerative slips such as spondylolisthesis.

Statistical Analysis

The characteristics of the population (e.g., sociodemographic) were described using means \pm standard deviation (SD) for continuous variables, and frequency for qualitative variables.

Ethical Considerations

Retrospective registry-based studies do not require ethics committee approval under French law. Patient data were extracted from the patient electronic record from the reference centre of rare diseases. Patient data were then collected in a database approved by the National Commission for Data Protection and Liberties (CNIL).

RESULTS

Patient Characteristics

Twenty-eight patients (27 female and one male) with a diagnosis of hEDS were selected and underwent a complete spine X-ray using EOS method and analysis (Table 1). The mean age at diagnosis was 30.10 (SD: ± 10.18 years; range 19.00–50.00 years). The mean BMI was 25.19 (SD: ± 4.88). Seventy-one percent (n=20/28) were employed at the time of diagnosis. Twenty-one percent (n=6/28) had at least one orthopedic surgery at the time of diagnosis and 7% (n=2/28) had three or more surgeries.

Prevalence of the 2017 Criteria for hEDS in the Patient Population

study evaluated retrospectively the This prevalence of the 2017 diagnostic criteria, which made it possible to confirm the diagnosis of hEDS. Prevalence of these 2017 criteria in the patient population are detailed in Table 2. Generalised joint hypermobility was found in 100.0% of patients. The mean Beighton score was 6/9 (SD: ±2). Sixty-four percent of patients were able to place their hands completely flat on the floor with their knees extended; 7.1% had done so in the past. Other clinical signs were found: bilateral piezogenic papules of the heels (53.6% of patients); moderate skin hyperextensibility (46.4%); unusually soft and velvety skin (42.9%); large unexplained striae (39.3%); arachnodactyly (25.0%); and a bilateral thumb sign (Steinberg's sign) and bilateral wrist sign (Walker's sign) in 17.9% and 7.1% of patients, respectively. Furthermore, 39.3% of patients presented with swan-neck deformities of the fingers, 17.9% presented with dental crowding and/or a high or narrow palate, and 14.3% presented with atrophic scars in at least two places. One patient (3.5%) presented with aortic root dilation and two patients (7.1%) presented with mitral valve prolapse on their ultrasound. One patient (3.5%) had an arm-span-to-height ratio ≥1.05 and one patient (3.5%) presented with recurrent and multiple abdominal hernias. No patients had pelvic, rectal, or uterine prolapse in their history (Table 2). Family history was found in 50.0% of patients.

Prevalence and Types of Scoliosis in the Patient Population

In this patient population, scoliosis was found in almost 29% (n=8/28). None of these patients had undergone spine surgery (Table 1). Among the 29% of patients with scoliosis, 12.5% (n=1/8) had a right thoracic form, 12.5% (n=1/8) had a left thoracic form, 50.0% (n=4/8) had a thoracolumbar form, and 25.0% (n=2/8) had a unique lumbar form. A mild form of scoliosis with Cobb angle \leq 19° was found in 87.5% of patients (n=7/8), 12.5% (n=1/8) had a moderate form (Cobb angle <29°), and none had a severe form (Cobb angle >30°). The mean Cobb angle was 13.6° (SD: ±3.5°).

Age, sex	BMI	BS	Ability to put hands flat on the floor	PI	SS	PT	Roussouly type	Type of scoliosis	Cobb angle
Patients with so	coliosis							•	
28, F	24.46	6	No	48	32	16	1 or 2	Left lumbar L1-L4	12°
25, F	23.19	5	Yes	45	38	4	3	Left thoracic T1-T6	10°
49, F	31.63	7	Yes	75	42	33	3	Right lumbar	10°
25, F	22.20	7	Yes	53	45	8	3	Right thoracic	13°
21, F	21.20	6	No	NA	NA	NA	NA	Left thoracolumbar	15°
36, F	32.47	6	Yes	89	37	52	3	Thoracolumbar	13°
20, F	19.71	5	Yes	49	NA	NA	NA	Thoracic and lumbar	15-16 and 22°
26, F	17.80	4	Yes	32	35	4	3	Thoracolumbar	14°
Patients with so	coliotic inflect	tion							
27, F	22.99	5	Yes	40	33	6	1 or 2	Right thoracic, left lumbar	NA
33, F	31.25	6	NA	44	34	10	1 or 2	Left thoracolumbar	NA
27, F	22.06	5.5	Yes	62	55	6	4	Left cervicothoracic	NA
19, F	23.15	6.5	Yes	56	51	5	4	Left thoracolumbar	NA
26, F	25.35	8	Yes	38	25	12	1 or 2	NA	NA
20, F	35.56	8	Yes	43	35	9	3	Left lumbar	NA
24, F	25.39	5	Yes	49	33	16	1 or 2	Right lumbar	NA
28, F	19.23	7	Yes	76	53	23	4	Left lumbar	NA
47, F	23.80	5.5	Yes	52	17	35	1 or 2	NA	NA
Patients withou	it spinal abno	, rmaliti	es					1	
21, F	24.51	8	No	27	28	-1	1 or 2	NA	NA
41, F	26.40	8	Yes	55	43	13	3	NA	NA
50, F	26.26	4	Yes	51	41	11	3	NA	NA
26, M	35.24	7	No	47	22	25	1 or 2	NA	NA
22, F	30.49	5	Yes	63	61	2	4	NA	NA
48, F	31.59	9	Yes	38	37	2	3	NA	NA
47, F	26.31	5.5	Yes	62	37	26	4	NA	NA
25, F	22.84	5	Yes	37	26	11	1 or 2	NA	NA
21, F	19,95	8	Yes	48	27	21	3	NA	NA
42, F	20,45	6	Yes	50	48	2	4	NA	NA
19, F	19,88	6	No	36	30	6	1 or 2	NA	NA

BS: Beighton score; EOS: EOS[®] X-ray (EOS imaging, Paris, France); F: female; M: male; NA: not assessed; PI: pelvic incidence; PT: pelvic tilt; SS: sacral slope.

Table 2: Prevalence of the 2017 diagnostic criteria for hypermobile Ehlers-Danlos syndrome and scoliosis in the patient population.

2017 criteria for hypermobile Ehlers-Danlos syndrome	Frequency				
Generalised joint hyperlaxity	100.0%				
Bilateral piezogenic papules of the heels	54.0%				
Positive family history	50.0%				
Moderate skin hyperextensibility	46.0%				
Unusually soft and velvety skin	43.0%				
Large unexplained striae	39.0%				
Swan-neck deformities of the fingers	39.0%				
Arachnodactyly	25.0%				
Bilateral thumb sign	18.0%				
Dental crowding and/or a high or narrow palate	18.0%				
Atrophic scars in at least two places	14.0%				
Mitral valve prolapse	7.0%				
Bilateral wrist sign	7.0%				
Aortic root dilation	3.5%				
Arm-span-to-height ratio ≥1.05	3.5%				
Recurrent and multiple abdominal hernias	3.5%				
Pelvic, rectal, or uterine prolapse	0.0%				
Frequency of spinal deformity and spine surgery					
Scoliotic inflection	32.0%				
Scoliosis	29.0%				
Spine surgery	0.0%				

In addition, 32.1% of the patients (n=9/28) presented with a simple scoliotic inflection and 39.3% (n=11/28) had neither scoliosis nor a scoliotic inflection (Table 1).

DISCUSSION

In this study, the authors evaluated the frequency and severity of scoliosis in patients with hEDS. Limited data regarding scoliosis in hEDS are currently available in the literature. Results showed that 29% of the patients with hEDS presented with scoliosis at the initial diagnosis. Scoliosis was not retained among the diagnostic criteria for hEDS in the 2017 classification. This choice could be challenged in light of other subjective criteria retained in this classification, the prevalence of which are identical or even lower. The question remains for the potential relationship between scoliosis, hypermobility, and hEDS, which was not the objective of this study.

Natural History of Scoliosis and the Impact of Joint Hypermobility

None of the patients had a severe form of scoliosis; most were benign. However, regarding natural history of scoliosis, it is critical to diagnose scoliosis early in order to ensure regular follow-up and appropriate management of hypermobile patients.

Idiopathic scoliosis is frequent, with an overall prevalence of 1-3% most often cited in the literature. ¹⁹ The female-to-male ratio ranges from 1.5:1 to 3:1 and increases substantially with increasing age.²⁰ The prevalence of generalised joint hypermobility among children and adolescents varies significantly (7-65%), depending on measurement method, sex, age, and ethnicity.²¹ Several studies suggested that generalised joint hypermobility is more common in subjects with scoliosis compared to a control population of patients of the same sex and age. A 2011 study by Czaprowski et al.,²² which observed 70 subjects with idiopathic scoliosis (59 girls and 11 boys), found generalised joint hypermobility in >50.8% of girls (n=30/59) and 54.5\% of boys (n=6/11) compared with a control group of healthy subjects (21.0% of girls and 16.0% of boys); this difference was significant.²² A second study by Czaprowski et al.⁹ in 2014 confirmed these data in 155 girls aged 9-18 years with idiopathic scoliosis compared to a control group of 201 girls without scoliosis. It was revealed that 23.2% of girls with scoliosis were hypermobile compared to 13.4% in the control group (p=0.02).⁹ However, joint hypermobility did not seem to influence the prognosis of scoliosis. In this study, there was no correlation between the presence of generalised joint hypermobility and the severity of scoliosis. There was no correlation between the measured Beighton score and the Cobb angle (p=0.93).⁹

A 2018 study carried out among 822 Turkish students (413 boys and 409 girls) with an average age of 12.2 years (SD: ±1.3 years) found hypermobility in 151 subjects, i.e., 18.6% of the study population (10.2% of girls and 8.4% of boys). Scoliosis was found in 5.2% of children (n=43/822), of which 23.2% (n=10/43) were hypermobile and 76.8% (n=33/43) were not. The presence of hypermobility was not associated with the presence of scoliosis.²³

Interestingly, a study by Haller et al.,²⁴ performed in 570 women with idiopathic scoliosis, looked at whether the Beighton score was predictive of scoliosis surgery. Between the different elements of the Beighton score measure, only the inability to put hands flat on the floor was predictive of the progression of scoliosis to surgery. Generalised joint hypermobility did not influence the risk of surgery, while lack of hypermobility (a Beighton score of 0/9) increased the risk of surgery in scoliosis. The authors specified that women

who could not put their hands flat on the floor had a 2.1-times greater chance of having surgery compared to those who could (p=0.001).²⁴ This single measure was useful and easy to perform for predicting the progression of idiopathic scoliosis. The hypermobility and flexibility of the spine could be, according to the authors, factors of better response to the brace, avoiding aggravation and need for surgery.

Scoliosis and Ehlers-Danlos Syndrome

EDS is one of the possible diagnoses to consider in the presence of unusual or secondary appearance scoliosis. Ligament laxity, postural abnormalities, and muscle weakness found in EDS could be factors favouring this scoliosis.²³ There are limited data available in the literature regarding the frequency and severity of scoliosis in the different forms of EDS.¹² In kyphoscoliotic EDS, scoliosis is secondary to severe muscle hypotonia and ligament laxity.¹¹ It appears early in infancy and is part of the diagnostic criteria. Molecular biology can confirm the diagnosis. In these early forms, the progression of scoliosis is severe and surgical treatment is often necessary.

In 2000, Stanitski et al.¹⁰ published a large series of patients affected by three different forms of EDS (hypermobile, classic, or vascular) in whom scoliosis was evaluated.¹⁰ Thirty-three percent of patients had scoliosis (30% of hEDS, 33% of vascular EDS, and 36% of classic EDS). Scoliosis therefore seems slightly more frequent in classic EDS, a feature already found in a previous publication according to the authors. No patient presented with scoliosis requiring surgery (Cobb angle >50°). The curves were mild-to-moderate in 71% of cases with hEDS. No significant correlation was found between back pain and the existence of scoliosis.¹⁰ Analysis of EOS spine X-ray of the patients confirmed the data available in the literature. Twenty-nine percent of patients with hEDS presented with mild-to-moderate scoliosis. No patient had a severe scoliosis. Interestingly, 32% of patients presented with a scoliotic inflection, not to be confused with scoliosis. This scoliotic inflection could be linked to an external phenomenon, such as inequality in length of the lower limbs or a tilting of the pelvis.

In adulthood, only 10% of cases of mild-tomoderate scoliosis have a risk of progression;²⁴ in theory, there is therefore very little risk of progression of scoliosis in the population of patients with hEDS. Even if joint hypermobility seems to influence the development of scoliosis, it seems that its presence is reassuring regarding the possible progression of scoliosis.²⁴ It also seems to be the case in the hypermobile adult population of this study, where no severe scoliosis requiring surgery was found. During clinical examination, the ability to put hands flat on the floor, assessed by the Beighton score and found in 75% (n=6/8) of patients with a scoliosis, also seems to be an interesting element in the prediction of the favourable evolution of scoliosis. Further studies are needed to determine if the ability to put hands flat on the floor could be a factor explaining the good response to brace treatment in hypermobile patients with moderate or progressive scoliosis, and whether this could explain the absence of severe scoliosis in patients with hEDS.

Scoliosis was not retained among the diagnostic criteria for hEDS in the 2017 classification. This choice can be challenged in light of other subjective criteria retained in this classification, the frequency of which are identical or even lower.

Study Limitations

This present study should be considered with certain limitations. Firstly, the study was performed using a small population size and it would therefore be necessary to assess the exact prevalence of scoliosis in a larger population of patients with hEDS. Secondly, prevalence was not compared with a group of patients presenting with only joint hypermobility. Currently, it is not possible to conclude if scoliosis could be linked to hypermobility itself or if it could be considered as an additional criterion in hEDS diagnosis. A comparative study with a control group should be considered. Moreover, it is worthy of note that hEDS is mainly seen in the female population in which scoliosis is much more frequent, meaning that some scoliosis could be linked to sex and not to disease itself.⁷ Thirdly, absence of genetic confirmation in hEDS makes the diagnosis difficult since it is mainly based on clinical examination plus some echographic features.

Highlighting the prevalence of scoliosis in patients with hEDS, this study supports the importance of considering screening for scoliosis in any patients meeting the criteria for hEDS. Regular follow-ups could avoid scoliosis progression by appropriate management. It seems fundamental, given the preponderance of females in the hEDS population, to be attentive to screening for scoliosis at the first signs of puberty. Repeated use of EOS makes it possible to monitor scoliosis until patients are adults.

CONCLUSION

This retrospective study showed that scoliotic inflections and scoliosis are frequent in patients with hEDS. Considering this observation, the authors recommend performing full spinal X-ray in all patients with hEDS at the time of initial diagnosis and regularly during follow-up.

References

- Castori M, Hakim A. Contemporary approach to joint hypermobility and related disorders. Curr Opin Pediatr. 2017;29(6):640-9.
- Malfait F et al. The 2017 international classification of the Ehlers-Danlos syndromes. Am J Med Genet Part C Semin Med Genet. 2017;175(1):8-26.
- Malfait F et al. Defective initiation of glycosaminoglycan synthesis due to B3GALT6 mutations causes a pleiotropic Ehlers-Danlos-syndromelike connective tissue disorder. Am J Hum Genet. 2013;92(6):935-45.
- Ritelli M et al. Expanding the clinical and mutational spectrum of recessive *AEBP1*-related classical-like Ehlers-Danlos syndrome. Genes (Basel).

2019;10(2):135

- Joseph AW et al. Characteristics, diagnosis, and management of Ehlers-Danlos syndromes: a review. JAMA Facial Plast Surg. 2018;20(1):70-5.
- Tinkle B et al. Hypermobile Ehlers-Danlos syndrome (a.k.a. Ehlers-Danlos syndrome Type III and Ehlers-Danlos syndrome hypermobility type): clinical description and natural history. Am J Med Genet C Semin Med Genet. 2017;175(1):48-69.
- Demmler JC et al. Diagnosed prevalence of Ehlers-Danlos syndrome and hypermobility spectrum disorder in Wales, UK: a national electronic cohort study and

case-control comparison. BMJ Open. 2019;9(11):e031365.

- Goldstein LA, Waugh TR. Classification and terminology of scoliosis. Clin Orthop Relat Res. 1973;93:10-22.
- Czaprowski D. Generalised joint hypermobility in caucasian girls with idiopathic scoliosis: relation with age, curve size, and curve pattern. ScientificWorldJournal. 2014;2014:370134.
- Stanitski DF et al. Orthopaedic manifestations of Ehlers-Danlos syndrome. Clin Orthop Relat Res. 2000;(376):213-21.

- Brady AF et al. The Ehlers-Danlos syndromes, rare types. Am J Med Genet Part C Semin Med Genet. 2017;175(1):70-115.
- Stern CM et al. Musculoskeletal conditions in a pediatric population with Ehlers-Danlos Syndrome. J Pediatr. 2017;181:261-66.
- The Ehlers-Danlos Society. hEDS diagnostic checklist. 2021. Available at: https://www.ehlers-danlos.com/ heds-diagnostic-checklist/. Last accessed: 14 January 2021.
- Bulbena A et al. Clinical assessment of hypermobility of joints: assembling criteria. J Rheumatol. 1992;19(1):115-22.
- Smits-Engelsman B et al. Beighton score: a valid measure for generalized hypermobility in children. J Pediatr. 2011;158(1):119-23.

- Juul-Kristensen B et al. Measurement properties of clinical assessment methods for classifying generalized joint hypermobility – a systematic review. Am J Med Genet Part C Semin Med Genet. 2017;175(1):116-47.
- 17. Naal FD et al. Validation of a selfreported Beighton score to assess hypermobility in patients with femoroacetabular impingement. Int Orthop. 2014;38(11):2245-50.
- Roussouly P et al. Classification of the normal variation in the sagittal alignment of the human lumbar spine and pelvis in the standing position. Spine (Phila Pa 1976). 2005;30(3):346-53.
- Negrini S et al. 2016 SOSORT guidelines: orthopaedic and rehabilitation treatment of idiopathic scoliosis during growth. Scoliosis Spinal Disord. 2018;13(1):1-48.

- Konieczny MR et al. Epidemiology of adolescent idiopathic scoliosis. J Child Orthop. 2013;7(1):3-9.
- Adib N et al. Joint hypermobility syndrome in childhood. A not so benign multisystem disorder? Rheumatology (Oxford). 2005;44(6):744-50.
- 22. Czaprowski D et al. Joint hypermobility in children with idiopathic scoliosis: SOSORT award 2011 winner. Scoliosis. 2011;6:22.
- 23. Bozkurt S et al. Hypermobility frequency in school children: relationship with idiopathic scoliosis, age, sex and musculoskeletal problems. Arch Rheumatol. 2019;34(3):268-73.
- 24. Haller G et al. Lack of joint hypermobility increases the risk of surgery in adolescent idiopathic scoliosis. J Pediatr Orthop Part B. 2018;27(2):152-8.

Musculoskeletal Brucellosis in Adults in the United Arab Emirates: A Retrospective Study

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Abstract

Introduction: Brucellosis is a zoonotic infection caused by the aerobic Gram-negative bacteria coccobacilli, and is considered a public health problem in the Mediterranean region and Arabian Peninsula. This paper studied the clinical characteristics of musculoskeletal brucellosis and the outcomes of treatment in Al Ain City, United Arab Emirates.

Method: A retrospective chart review study was conducted at Tawam Hospital over seven years: January 2009–January 2016. Risk factors for brucellosis, musculoskeletal (MSK) manifestations, duration of Brucella infection (acute, subacute, chronic), and treatment were studied.

Results: A total of 99 patients were diagnosed with brucellosis during the study period; the mean age was 44 years, the majority were males (71%), and the male to female ratio was 3:1. The most common risk factor for Brucella infection in the cohort was drinking raw milk (43.4%). Fever was the most common presenting symptoms (93%), followed by arthralgia, fatigue, and loss of appetite in 35, 21, and 14%, respectively. The clinical manifestations of brucellosis in the cohort were MSK involvement (30%), hepatitis (17%), epididymo-orchitis (2%), and endocarditis (1%). Thirty percent of patients (n=30) had MSK-specific symptoms and only one-third (n=10) had confirmatory positive radiographic findings. The majority of patients had lumbar and sacroiliac joint involvement. Most of the patients received antibiotics for a 4–8-week duration and the overall relapse rate of Brucella infection was 10%.

Conclusion: This study demonstrates that MSK involvement is a common manifestation in brucellosis, occurring in one-third of the cases. The index of suspicion should be high in brucellosis-endemic countries for early recognition and treatment.

INTRODUCTION

Brucellosis is a zoonotic infection, historically known as Maltese fever, caused by aerobic Gram-

negative coccobacilli, which was first identified by Sir David Bruce in 1887.¹ Worldwide, the true prevalence of brucellosis is unknown; however, it is considered a public health problem in the Mediterranean region, Arabian Peninsula, India, Mexico, and part of the USA. Pappas et al.² estimated the global incidence of brucellosis to be more than half a million cases annually, and the incidence in endemic disease areas to be from <0.01->200 cases per 100,000 of the population. In the United Arab Emirates (UAE), the total brucellosis notification rate between 2010 and 2015 was estimated to be 3.3 per 100,000 people per year in Abu Dhabi city, the capital of the UAE.³

Brucella is a facultative intracellular bacterium that infects animals and has various different species: Brucella melitensis (goats, sheep, camels), B. abortus (bovine, cattle, camels), B. suis (swine, cattle), B. canis (dogs), B. ovis (ram, sheep), and *B. neotomae* (desert rats).¹ An epidemiological study measuring the prevalence of Brucella in 6,126 livestock in Abu Dhabi reported higher prevalence in sheep and goats (8.4%) compared to camels (4.4%).⁴ Risk factors of Brucella infection include drinking unpasteurised animal milk products, direct contact with infected animals, inhalation of aerosolised particles, and occupational and work-related diseases (shepherds, abattoir workers, veterinarians, dairy-industry professionals, and microbiologic laboratory workers).^{1,5,6} Clinical manifestations of Brucella infection vary depending on the organ involved. Common presenting symptoms are fever (78%), chills (45%), sweats (54%), fatigue (39%), weight loss (26%), arthralgia (65%), and abdominal pain (19%) due to hepatomegaly or splenomegaly.⁷ Brucella infection is classified based on the time of clinical presentation as acute brucellosis (0-2 months), subacute brucellosis (2-12 months), and chronic brucellosis (>12 months). The laboratory findings might reveal leukocytosis (9%), leukopenia (10%), anaemia (40%), thrombocytopenia (10%), elevated liver enzymes (24%), and inflammatory markers (>50%; C-reactive protein [CRP], and erythrocyte sedimentation rate [ESR]). The majority of patients (94%) will have a positive Brucella titre (tube agglutination test) and bacteraemia may be encountered in approximately 11% of cases.⁵

Musculoskeletal (MSK) involvement of brucellosis represents 10–85% of reported clinical manifestations such as peripheral arthritis, bursitis, sacroiliitis, osteomyelitis, spondylitis, and paraspinal abscess.^{8,9} Spinal brucellosis is common in the elderly and predominately affects the L4–L5 spinal segment of the lumbar spine, followed by thoracic and cervical spine.^{9,10} Diagnosis of osteoarticular brucellosis requires laboratory tests to confirm Brucella infection and imaging studies. MRI produces a high yield of images that detect radiological features of spinal brucellosis. There is no prior study, to the authors knowledge, describing the clinical manifestations of MSK brucellosis in the UAE; therefore, a retrospective study was conducted, with the aim of identifying the clinical characteristics of MSK brucellosis and outcomes of treatment in Al Ain, UAE.

METHODS

A retrospective chart review study was conducted at Tawam hospital (tertiary hospital, Al Ain, UAE) over seven years (January 2009-January 2016). Ethical approval was obtained from Tawam Human Research Ethics Committee (T-HREC). Adult patients (>16 years of age) diagnosed with brucellosis during the study period were involved. The diagnosis of brucellosis was confirmed by either a positive Brucella titre of >1:160 and/or positive blood culture. Citizens were described as 'nationals' and non-citizens as 'non-nationals'.

Demographic, clinical, and laboratory data were collected and analyzed using descriptive analysis. Risk factors for Brucella infection and clinical presenting symptoms were studied. Patients were classified into subgroups according to arthritis types, either peripheral or axial. Brucella infection was classified based on the time of clinical presentation as acute brucellosis (0-2 months), subacute brucellosis (2-12 months), and chronic brucellosis (>12 months).⁵ Relevant blood tests were included, and confirmatory radiographic imaging was obtained in all patients with MSK complaints (plain radiograph/X-ray, CT scan, or MRI). Treatment of MSK brucellosis and outcomes were identified.

RESULTS

A total of 99 patients were diagnosed with brucellosis during the study period. The mean age was 44 years, the majority were males (71%), and the male to female ratio was nearly 3:1. Twothirds of patients were nationals (63 out of 99; 63.3%). The most common factor for Brucella infection in the cohort was drinking raw milk (43 out of 99; 43.4%); the remaining are summarised in Table 1.

Fever was the most common symptom and accounted for 93% of all patients, followed by arthralgia, fatigue, and loss of appetite in 35, 21, and 14%, respectively. Brucella infection was confirmed in all patients based on elevated Brucella titres (*B. melitensis* and *B. abortus*) of >1:160. Brucella bacteraemia (positive blood culture) was identified in only 57 patients. Other laboratory investigations revealed leukocytosis (white blood cell count >11x10⁹/L in 10 out of 99;

10.1%), anaemia (30 out of 99; 30.3%), abnormal liver enzymes (47 out of 99; 47.5%), and elevated CRP (83 out of 99; 83.8%). Systemic complications of brucellosis in the cohort were MSK involvement (30%), hepatitis (17%), epididymo-orchitis (2%), and endocarditis (1%).

Of the patients with MSK involvement (n=30), only one-third (n=10) had confirmatory positive radiographic findings. The most common joint involved was the lumbar spine, accounting for 34.5% (n=10) of the cases; followed by sacroiliac and shoulder joints, each accounting for 23.3% (n=7); and then the hip and knee joints, accounting for 13.3% (n=4).

Table 1: Demographic of patients (n=99), risk factors for Brucella infection and the presenting symptoms.

	N=99
Demographics	•
Male	71
Female	28
Risk factors	
Farm contact with animals	18
Raw milk drinking	43
Boiled milk drinking	3
Fresh cheese ingestion	1
Ingestion of raw meatballs	1
Laboratory worker	0
Unknown transmission	33
Symptoms	^ ^
Arthralgia	35
Arthralgia Fatigue	35 21
Arthralgia Fatigue Back pain	35 21 23
Arthralgia Fatigue Back pain Fever	35 21 23 94
Arthralgia Fatigue Back pain Fever Sweating	 35 21 23 94 15
Arthralgia Fatigue Back pain Fever Sweating Headache	 35 21 23 94 15 10
Arthralgia Fatigue Back pain Fever Sweating Headache Weight loss	 35 21 23 94 15 10 10
Arthralgia Fatigue Back pain Fever Sweating Headache Weight loss Loss of appetite	 35 21 23 94 15 10 10 15
Arthralgia Fatigue Back pain Fever Sweating Headache Weight loss Loss of appetite Nausea	35 21 23 94 15 10 15 3
Arthralgia Fatigue Back pain Fever Sweating Headache Weight loss Loss of appetite Nausea Vomiting, abdominal pain	35 21 23 94 15 10 15 3 10

The radiographic imaging using X-ray and MRI were positive in 10 patients with MSK brucellosis (9 males, 1 female). Five patients had Brucella bacteraemia and all had high Brucella titres, ranging from 1:320 to 1:10,240. The majority of patients had lumbar and sacroiliac joint involvement, with radiographic imaging findings such as disc involvement, vertebral body destruction or abscess, unilateral sacroiliitis, and osteomyelitis. Two patients had septic arthritis related to Brucella infection involving the hip and knee joints, with positive radiological findings of joint effusion. The majority of patients with MSK brucellosis who had positive radiographic changes were diagnosed during the acute phase (90%; n=9) (Table 2, Figure 1).

Details of brucellosis treatment were available in 88 out of 99 patients. All patients received combination therapy with at least two antibiotics. A third agent, mostly an aminoglycoside, was added to treatment in 27 patients (30.6%) for MSK involvement. The most common antibiotics used were doxycycline (87 out of 88; 98.8%), rifampin (76 out of 88; 86.3%), gentamicin (20 out of 88; 22.7%), trimethoprim/sulfamethoxazole (14 out of 88; 15.9%), and ciprofloxacin (5 out of 88; 5.6%). The duration of therapy varied depending on severity and organ involvement. A majority of patients received 4-6 weeks of antibiotics (64 out of 88), followed by 8 weeks of therapy in 15 patients; only two patients required prolonged antibiotic treatment of more than 8 weeks. Surgical therapy was provided for patients (n=2) with MSK brucellosis for draining vertebral abscess, septic arthritis, and discitis. The overall relapse rate of Brucella infection in the cohort (n=99) was 10%.

DISCUSSION

The burden of Brucella infection and its multisystem involvement is under-reported in some endemic areas. In a meta-analysis of the clinical manifestations of human brucellosis, adult males were affected in 56% of studies, and MSK complaints were common presenting symptoms (65% arthralgia, 47% myalgia, and 45% back pain). The potential risk factors identified for brucellosis were consumption of unpasteurised dairy products in 64% of the studies, followed by contact with livestock in 42%, occupational exposure in 6%, and positive family history of

brucellosis in 20% (ranging from 17% to 46%). Interestingly, some studies advocated for Brucella screening among families where one member had been diagnosed, due to the possibility of sharing the same risk factors.^{5,7,11}

This study reports 99 cases of confirmed brucellosis; the mean age of patients was 44 years, and men were three times more affected than women. This demonstrates an increased risk to those men involved in animal husbandry. The most significant risk factor was consumption of unpasteurised milk. These findings are consistent with a study conducted in the same region, in which the disease affected mostly men with a mean age of 40 years and where the risk factors constituted of consumption of unpasteurised milk and dairy products.³ This may explain the importance of cultural and environmental impact.

The presenting symptoms of Brucella infection nonspecific, and many cases are were identified during work-up for fever of unknown origin. Differential diagnosis often includes other infectious and non-infectious chronic inflammatory conditions. Pourbagher et al.¹⁰ noted that fever, fatigue, loss of appetite, nausea, and diarrhoea were the main reported symptoms in the acute phase, while weight loss and palpitation were observed during the subacute phase and the osteoarticular manifestation during the chronic stage.¹⁰ Similarly, in this study, fever was the most common presenting symptom in 93% of patients, followed by arthralgia, fatigue, and loss of appetite in 35, 21, and 14%, respectively. In contrast, osteoarticular involvements in this cohort were diagnosed during the acute phase. Physician education, a higher level of suspicion, and easy availability of serological testing and positive blood cultures may account for this finding.

In this cohort, 30 adult patients were identified with MSK-specific symptoms; one-third had confirmatory positive radiographic findings as described earlier. The reported MSK involvement in different studies varied; along with variation at the affected site, rheumatic complaints were reported in 20-85% of brucellosis cases.¹²⁻¹⁴ In a study conducted in Turkey with 251 patients, 45.4% (n=114) of cases reported osteoarticular involvement, with the most common site being the sacroiliac joint (28.3%) followed by lumbar spine (10.4%).¹⁰

Table 2: Clinical characteristics of patients with musculoskeletal brucellosis and positive radiological findings.

	Age	Gender	Stage	Blood culture	Brucella titre*	Complications	Radiographic changes
Case 1	33	Male	Acute	Negative	1:640	Right	X-ray SIJ: no abnormalities
						sacroiliitis	MRI SIJ: unilateral right sacroiliitis
Case 2	89	Male	Acute	Positive	1:10,240	Lumbar abscess collection	X-ray lumbar spine: intervertebral space narrowing L1–L2, L3–L4, and L5–S1
						Hepatitis	MRI lumbar spine: collection in the lumbar spine
Case 3	80	Male	Acute	Negative	1:1,280	Lumbar discitis and	X-ray lumbar spine: vertebral body destruction L4/5
						Osteomyeittis	MRI lumbar spine: L4/L5 disc and endplates signal abnormality, highly suspicious of discitis and osteomyelitis
Case 4	35	Male†	Acute	Negative	1:2,560	Left knee monoarthritis (septic)	X-ray left knee: no abnormalities MRI left knee: effusion
Case 5	20	Male	Acute	Positive	1:2,560	Septic right-sided sacroiliitis	X-ray SIJ: no abnormalities MRI SIJ: mild right-sided septic arthritis with small collection underneath the anterior sacroiliac ligament
Case 6	35	Male	Acute	Positive	1:2,560	Lumbar disc involvement Hepatitis	X-ray lumbar spine: disc involvement MRI lumbar spine: disc involvement L5/S1, nerve compression
Case 7	28	Female	Acute	Negative	1:640	Left hip monoarthritis (septic) Osteomyelitis	X-ray hip: no abnormalities MRI left hip: joint effusion
Case 8	18	Male	Acute	Positive	1:10,240	Septic	X-ray SIJ: no abnormalities
						sacroiliitis	MRI SIJ: left sacroiliitis
Case 9	71	Male	Subacute	Negative	1:640	Lumbar discitis	X-ray lumbar spine: no abnormalities
						riepaulis	MRI lumbar spine: disc involvement, discitis D1/L1 and L3/L4
Case 10	31	Male	Acute	Positive	1:320	Sacroiliac sclerosis	X-ray SIJ: left SIJ sclerosis

*both (*Brucella abortus* and *B. melitensis*). †expatriate. SIJ: sacroiliac joint.



Figure 1: Positive MRI findings in selected cases as described in Table 2.

In another study from Saudi Arabia, with 84 cases of brucellosis, MSK complication was the most common symptom and 64% were found to have either peripheral arthritis, sacroiliitis, or spondylitis.¹⁵ In this study, almost 80% (n=8) of patients had axial involvement affecting lumbar and sacroiliac joints with positive radiographic imaging (disc involvement, discitis, vertebral body destruction. unilateral sacroiliitis). Peripheral involvement is less common compared with axial involvement. Furthermore, Buzgan et al.⁵ reported osteoarticular Brucella involvement in 260 cases out of 1,028 patients (25.3%), involving peripheral arthritis (56.5%), sacroiliitis (24.6%) (unilateral in 51 cases), spondylitis (12.3%), and paraspinal abscess (3.5%). They received various therapeutic regimens for a duration of approximately 6-12 weeks and the overall relapse rate was 4.7% (in osteoarticular involvement: 8.5%).5

The imaging modalities used to diagnose MSK Brucella involvement varies in published literature. For peripheral arthritis, ultrasound can detect effusion. In one study, 5.2% of patients presenting with arthralgia, diagnosed with bursitis (13 out of 251 Brucella-infected cases) using ultrasound, had negative synovial fluid culture.¹⁰ Brucellosis with sacroiliac joint involvement can be diagnosed using a plain radiograph (late findings), radionuclide bone scintigraphy (bone scan), or MRI. It is estimated that two-thirds of cases will have unilateral sacroiliitis and only one-third will be bilateral. A bone scan showed increased uptake in the sacroiliac joints.^{5, 9, 10} Radiographs in vertebral brucellosis may reveal abnormalities in vertebral endplates, associated with irregularities and narrowing intervertebral disk spaces in subacute and chronic Brucella infection. MRI is very sensitive for detecting spondylodiscitis, vertebral destruction, spinal stenosis/abscess,

and osteomyelitis. It is fundamental to rule out tuberculosis infection, which has predilection to same articular involvement (Pott's Disease) with chest radiographs, QuantiFERON tests, and acidfast bacilli stain and culture on biopsy.^{9,10}

The aim of medical therapy in cases of brucellosis is to prevent complications and relapse and to control the acute illness. Monotherapy and short duration of <4 weeks are not recommended in the treatment of brucellosis due to high risk of treatment failure and relapse. In a metaanalysis, the combination of streptomycin for 2-3 weeks plus doxycycline for 6 weeks had a lower failure rate than doxycycline plus rifampicin for 6 weeks.^{16,17} Other antibiotics, such as quinolones (e.g. ciprofloxacin and ofloxacin), aminoglycosides (e.g. gentamicin), and trimethoprim/sulfamethoxazole, have been used along with other combinations and have shown variable effects.^{8,18} The optimal duration of therapy for MSK brucellosis is not well established, but prolonged therapy is required for complicated

cases. In the current cohort, all patients were on a combination of two antibiotics (doxycycline plus rifampicin regimen being the most common) and patients with MSK involvement required the addition of aminoglycosides (gentamicin). The majority of the patients received antibiotics for a duration of 4–8 weeks. The overall relapse rate in our cohort was 10%, of which three cases were related to noncompliance to medication, and of which one case had an earlier diagnosis of MSK brucellosis. Treatment-related complications were not reported in the study.

CONCLUSION

This study has demonstrated that MSK manifestation in brucellosis accounts for onethird of the cases. The index of suspicion should be high in brucellosis-endemic countries. Clinical symptoms, risk factors, specific laboratory findings, and clinical images are important for early recognition and treatment.

References

- Godfroid J et al. From the discovery of the Malta fever's agent to the discovery of a marine mammal reservoir, brucellosis has continuously been a re-emerging zoonosis. Vet Res. 2005;36(3):313-26.
- 2. Pappas G et al. The new global map of human brucellosis. Lancet Infect Dis. 2006;6(2):91-9.
- Al Shehhi N et al. Human brucellosis in the Emirate of Abu Dhabi, United Arab Emirates, 2010-2015. BMC Infect Dis. 2016;16(1):558.
- Mohammed MA et al. Sero-prevalence and epidemiology of brucellosis in camels, sheep and goats in Abu Dhabi Emirate. Int J Anim Vet Adv. 2013;5(2):82-6.
- Buzgan T et al. Clinical manifestations and complications in 1028 cases of brucellosis: a retrospective evaluation and review of the literature. Int J Infect Dis. 2010;14(6):e469-78.
- 6. Musallam II et al. Systematic review of brucellosis in the Middle East: disease frequency in ruminants and humans and risk factors for human infection.

Epidemiol Infect. 2016;144(4):671-85.

- Dean AS et al. Clinical manifestations of human brucellosis: a systematic review and meta-analysis. PLoS Negl Trop Dis. 2012;6(12):e1929.
- Alp E, Doganay M. Current therapeutic strategy in spinal brucellosis. Int J Infect Dis. 2008;12(6):573-7.
- 9. Chelli Bouaziz M et al. Spinal brucellosis: a review. Skeletal Radiol. 2008;37(9):785-90.
- Pourbagher A et al. Epidemiologic, clinical, and imaging findings in Brucellosis patients with osteoarticular involvement. AJR Am J Roentgenol. 2006;187(4):873-80.
- Sofian M et al. Screening of family members of patients with acute brucellosis in an endemic area of Iran. Iran J Microbiol. 2013;5(3):215-9.
- Ulu-Kilic A et al. Update on treatment options for spinal brucellosis. Clin Microbiol Infect. 2014;20(2):075-82.
- 13. Geyik MF et al. Musculoskeletal

involvement of brucellosis in different age groups: a study of 195 cases. Swiss Med Wkly. 2002;132(7-8):98-105.

- Esmaeilnejad-Ganji SM, Esmaeilnejad-Ganji SMR. Osteoarticular manifestations of human brucellosis: a review. World J Orthop. 2019;10(2):54-62.
- Bukharie HA. Clinical features, complications and treatment outcome of brucella infection: ten years' experience in an endemic area. Trop J Pharm Res. 2009;8(4):303-10.
- 16. Franco MP et al. Human brucellosis. Lancet Infect Dis. 2007;7(12):775-86.
- Yousefi-Nooraie R et al. Antibiotics for treating human brucellosis. Cochrane Database Syst Rev. 2012;10(10):CD007179.
- Pappas G et al. Treatment of brucella spondylitis: lessons from an impossible meta-analysis and initial report of efficacy of a fluoroquinolone-containing regimen. Int J Antimicrob Agents. 2004;24(5):502-7.

Prevalence of Thromboembolic Complications in COVID-19 Infection: A Systematic Review and Meta-Analysis

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Abstract

Introduction: The coronavirus disease (COVID-19) infection is proved to be involved in the onset of thromboembolism episodes. This study aims to evaluate the prevalence of thromboembolic complications in patients with COVID-19 from March until May 2020.

Methods: A literature review was conducted in MEDLINE (via PubMed), Scopus, Embase, Cochrane, and CINHAL without any language and date of publication restriction (Prospero registration number CRD42020186925). The inclusion criteria were as following: 1) patients with diagnosis of COVID-19; 2) occurrence of thromboembolic event, and 3) patients older than 18 years of age.

A multi-variable random effects model was computed accounting for correlations among outcomes by considering a heterogeneous compound symmetry covariance matrix.

Results: Observational studies included 2,442 participants from 268 to 7,999 participants per study, 1,014 (41.52%) were male and 825 (33.78%) were female. The multi-variable pooled event rate of acute myocardial infarction was rare, estimated to be 0.03 (95% confidence interval [CI]: 0.00–0.07; p=0.23); this is also true for the meta-analytical estimate of disseminated intravascular disease which was 0.04 (95% CI: 0.00–0.08; p=0.03). Conversely, other events were found to be more frequent. Indeed, the pooled proportion of pulmonary embolism was 0.14 (95% CI: 0.08–0.20; p<0.001), while the venous thromboembolic event rate is 0.15 (95% CI: 0.09–0.30; p=0.04). The pooled intrahospital mortality rate was equal to 0.12 (95% CI: 0.08–0.16; p<0.001).

Conclusions: Thromboembolic events, particularly venous thromboembolic event rate and pulmonary embolism, are a frequent complication in patients hospitalised with COVID-19. These findings suggest that the threshold for clinical suspicion should be low to trigger prompt diagnostic

testing and that evaluation of therapeutic treatment should be considered in patients in intensive care units with COVID-19.

INTRODUCTION

The novel coronavirus disease (COVID-19) has posed an unprecedented threat to global healthcare systems. The case fatality rate has been estimated to be as high as 15% in some countries.¹ The reason for high mortality rates of the new coronavirus infection is still unclear.

Clinical manifestations may vary from asymptomatic patients to acute respiratory distress syndrome, shock, and multi-organ failure associated with an increased risk of death.² Coagulation abnormalities have been reported since the start of the pandemic. Some authors supposed that these abnormalities might be similar to those reported in the previous severe acute respiratory syndrome coronavirus (SARS-CoV-2) infections. Chong et al.³ reported an incidence of 11.4% for pulmonary embolism (PE) and 20.5% for deep venous thrombosis (DVT) in SARS-CoV-2-infected patients.³ The clinical course of COVID-19 infection is often accompanied systemic inflammation. endothelial by dysfunction, and coagulation activation, which may evolve into overt disseminated intravascular coagulopathy (DIC).⁴ Moreover, the systemic activation of blood coagulation and pulmonary thromboinflammation with local vascular damage caused by COVID-19 may increase the risk of venous thromboembolism (VTE) and pulmonary artery thrombosis.5

From a pathogenetic point of view, the mechanism of hypercoagulability in patients with COVID-19 infection is quite clear;⁶ however, there are still no clear data on the incidence of those episodes in patients with COVID-19 despite the numerous reports and studies related to thromboembolic episodes.

Better understanding of COVID-19-related thromboembolic risk will help to optimise diagnostic strategies and guide the design and conduction of randomised controlled trials on thromboembolic prevention.

Thus, the aim of this meta-analysis was to estimate the prevalence of thromboembolic complications in patients admitted for COVID-19 from March until May of 2020. As the disease and its treatments are rapidly evolving, this meta-analysis represents events during the first and the beginning of the second wave of COVID-19 infection.

MATERIALS AND METHODS

Study Design

This is a systematic review and meta-analysis. The authors followed the Preferred Reporting Items for Systematic Reviews and Metaanalyses (PRISMA) and the Meta-analysis of Observational Studies in Epidemiology (MOOSE) reporting guidelines. The study was registered in Prospero with the number CRD42020186925. Research strategy was developed according to the Population, Intervention or exposure, Comparison, and Outcomes (PICO) study design model; these factors were patients with COVID-19, thromboembolic event, none, and incidence of thromboembolic events, respectively. The research strategy was then adapted according to the specific characteristics of each database.

Data Sources and Search

The literature review was conducted in MEDLINE (via PubMed), Scopus, Embase, Cochrane and CINAHL without any language and date of publication restriction. The following search terms were used and adapted in each database with proper Boolean operator: COVID-19, severe acute respiratory syndrome coronavirus-2, SARS-CoV-2, thrombosis, thromboembolism, cerebrovascular accident, myocardial infarction, hypercoagulability, heart attack, coronary disease, ischaemic heart disease, coronary heart disease, vascular disease, and cardiovascular disorder. The reference list of the retrieved studies was also searched. The last update was made on the 7th of May 2020.

Eligibility Criteria

The inclusion criteria were as follows: 1) patients with diagnosis of COVID-19; 2) occurrence of thromboembolic event (without restrictions to the damaged organ); and 3) patients older than 18 years of age. There was no restriction in the design of the studies included. Studies evaluating the outcomes of interest postmortem were excluded.

Study Selection

As reported in Figure 1, the study selection procedure was performed according to PRISMA guidelines.⁷ The the software Covidence⁸ was used in all the phases of data collection and extraction. Two blinded reviewers screened the articles in each phase. In each stage, the disagreements between the reviewers were solved by another senior expert in the field.

Data Extraction

For each article, two reviewers extracted the following data: country, study design, author, and patient population characteristics (i.e., age, sex, comorbidities, laboratory exam results,

use of invasive mechanical ventilation, use of antithrombotic medications, ward, length of stay, mortality, type of thromboembolic event occurred, and number of events).

Outcomes

The outcome of interest was the prevalence of both arterial and venous thromboembolic events in patients with COVID-19. The authors included prospective and retrospective studies, case series and case reports in order to better understand the dimension of the problem.

Quality Assessment

The risk of bias was evaluated by two independent reviewers. The Joanna Briggs Institute Critical Appraisal tools were used for quality assessment, according to the design of the study⁹ (e.g., prospective studies, prevalence data, case report, and case series).



Figure 1: PRISMA flow chart of retrieved articles.

Agreement

Cohen's Kappa was used to evaluate the interrater agreement among two reviewers in the full-text selection. The percentage of agreement was 56.98%. This could be explained by the large difference experience in metanalysis between the operators. A third expert reviewer reconciliated the disagreement between operators.

Data Synthesis

A multi-variate random effects model was computed accounting for correlations among outcomes by considering a heterogeneous compound symmetry covariance matrix. The multi-variate I² heterogeneity was also calculated following the approach proposed by Jackson et al.¹⁰ A multi-level random effect meta-regression model was used to deal with heterogeneity $(|^2>75\%)$. accounting also for correlation among the outcomes. Restricted cubic spline was considered for nonlinear effects. The estimates obtained with the multi-level random effect models have been adjusted by using, as moderators, the geographic areas (Europe and China), the study design (retrospective and prospective), and the median age of the study participants.

The univariate pooled random effect metaanalysis, together with the 95% confidence interval (CI), was also estimated for comparison purposes.

The incidence of discharge pooled estimate was computed using an incidence rate random effect meta-analysis calculating the events over the person time (length of hospitalisation) for each study.

Results and study level estimates were represented in a forest plot. The publication bias has been assessed via funnel plot graphical representation. Correlation matrix was computed among outcomes event rates with a 95% CI. Only endpoints reporting more than two pairwise comparisons have been computed for the correlation matrix. The analyses were conducted using R 3.6.2,¹¹ and rms¹² and metafor¹³ packages.

RESULTS

The search identified 2,110 studies. After title, abstract, and full-text screening, 38 studies were included in this review. Eighteen out of 26 studies were case reports and eight were case series. The remaining studies included in the meta-analysis were observational, eight were retrospective and six were prospective (Figure 1). The studies included in the metanalysis are shown in Table 1.¹⁴⁻⁵¹

Descriptive Characteristics of the Studies Included in Quantitative Analysis

Observational studies included 2,442 participants ranging from 26-799^{18,16} participants per study, of these 1,014 (41.52%) were male and 825 (33.78%) were female. They were mainly located in China^{16,22,27,52} and in France,^{17-19,21,23,25} the mean age reported was 49-70 years. Comorbidities were reported in 12 studies, 740 (30.30%) patients had at least one comorbidity (Table 1). Most patients included in the retrieved studies were admitted to the intensive care unit, except for those in the studies by Arachchillage et al.14 and Grillet et al.¹⁷ Antithrombotic therapy was routinely used in seven studies. They all used low-molecular weight heparin but with different dosage depending on the therapeutic or prophylactic usage. Only one study, by Llitjos et al., compared prophylactic versus the therapeutic the usage of anticoagulant¹⁸ in patients in ICU. The thromboembolic events and the number of events for each study are reported in Table 1. The considered events were grouped in the following categories: pulmonary embolism, venous thromboembolism, acute myocardial injury, arterial thromboembolism, and disseminated intravascular disease. PE was the most frequently reported event.17,20,21,23-26

Descriptive Characteristics of the Studies Included in Qualitative Analysis

Case report and case series studies are reported in Table 1. These retrieved studies reported the data of 56 participants overall (40 males [71.42%] with a median age of 60.53 years). These studies were mainly located in Italy^{28-30,35,38,47} and the USA.^{39,43,48-50,53}
Table 1: Descriptive characteristics of retrieved studies.

Author (year)	Country	Age (mean)*	N	Comor- bidities	D-dimer (ng/mL)	Fibrino- gen (g/L)	IMV (N)	Sex	Antithrom- botic	Ward	Type event (N)
Retrospective c	ohort stud	ies									
Arachchillage DRJ et al. ¹⁴ (2020)	China	54.1	183	75	66,000.00	4.55	NA	98 M, 85 F	NA	ICU	DIS (16) D (21)
Cattaneo M et al. ¹⁵ (2020)	Italy	70.0	64	NA	458.00	4.76	11	35 M, 29 F	NA	ICU	LOS (9)† VT (0)
Chen T et al. ¹⁶ (2020)	China	62.0	799	133	1,100.00	NA	119	171 M, 103 F	NA	ICU	D (113) DIS (21) DI (161) LOS (5) ⁺
Grillet F et al. ¹⁷ (2020)	France	66.0	100	92	NA	NA	34	30 M, 70 F	NA	NA	PE (23)
Llitjos JF et al. ¹⁸ (2020)	France	68.0	26	NA	1,750.00	699.00	26	6 M, 20 F	LWMH	ICU	PE (6) D (3) VT (18)
Lodigiani C et al. ¹⁹ (2020)	Italy	66.0	388	183	5,633.07	NA	NA	124 M, 264 F	LWMH	ICU/ ward	D (92) PE (10) DIS (8) LOS (10) [†] AMI (4) VT (4) CS (9)
Middeldorp S et al. ²⁰ (2020)	Nether- lands	61.0	173	18	1,100.00	NA	NA	130 M, 68 F	LWMH	ICU/ ward	D (38) VT (39) T (2) PE (13)
Poissy J et al. ²¹ (2020)	France	57.0	107	NA	NA	NA	67	13 M, 44 F	LWMH	ICU	PE (57) D (15)
Prospective coh	ort studies	5									
Cui S et al. ²² (2020)	China	59.9	81	33	NA	NA	NA	37 M, 44 F	NA	ICU	
Helms J et al. ²³ (2020)	France	68.0*	77	77	2,270.00	699.00	NA	63 M, 14 F	LWMH	ICU	PE (9) DI (36) AMI (0) VT (0) D (10)

Klok FA et al. ²⁴ (2020)	Nether- lands	64.0	184	75	NA	NA	45	139 M, 45 F	LWMH	ICU	PE (25) DI (22) AMI (0) VT (3) D (23) AT (3)
Leonard-Lo- rant I et al. ²⁵ (2020)	France	64.0	106	NA	NA	7.00	NA	70 M, 36 F	LWMH	ICU	PE (32) LOS (24) [†]
Tavazzi G et al. ²⁶ (2020)	Italy	68.0	54	NA	NA	657.00	54	44 M, 10 F	LWMH	ICU	PE (2) D (1) AMI (1) VT (8)
Wei JF et al. ²⁷ (2020)	China	49.0	101	54	NA	NA	11	54 M, 47 F	NA	ICU	D (3) LOS (5) [†] AMI (16)
Case reports											
Audo A et al. ²⁸ (2020)	Italy	59.0	1	0	NA	NA	1	М	NA	ICU	PE
Cellina M et al. ²⁹ (2020)	Italy	60.0	1	1	5,411.00	NA	NA	М	NA	NA	PE
Danzi GB et al. ³⁰ (2020)	Italy	75.0	1	0	21,000.00	NA	NA	F	NA	NA	PE
de Barry O et al. ³¹ (2020)	France	79.0	1	0	NA	NA	NA	F	NA	NA	VT
Dominguez-Er- quicia P et al. ³² (2020)	Spain	64.0	1	0	749.00	NA	NA	М	NA	NA	AI
Fabre O et al. ³³ (2020)	France	45.0	1	1	NA	NA	1	F	NA	ICU	PE
Foch E et al. ³⁴ (2020)	France	50.0	1	0	3,020.00	NA	NA	М	NA	NA	PE
Giacomelli E et al. ³⁵ (2020)	Italy	67.0	1	1	10,482.20	432.00	NA	М	LMWH	NA	AT
Harsch IA et al. ³⁶ (2020)	Germa- ny	66.0	1	NA	14,903.00	NA	NA	F	NA	NA	PE
LeBerre A et al. ³⁷ (2020)	France	71.0	1	NA	17,280.00	NA	NA	М	LMWH	NA	AT, PE
Martinelli I et al. ³⁸ (2020)	Italy	17.0	1	NA	16,400.00	602.00	NA	F	LMWH	Mater- nity hub	PE
Moshayedi P et al. ³⁹ (2020)	USA	80.0	1	1	NA	NA	NA	М	NA	NA	IS, MI
Rotzinger DC et al. ⁴⁰ (2020)	Switzer- land	75.0	1	0	NA	NA	NA	М	LMWH	NA	PE

Sulemane S et al. ⁴¹ (2021)	UK	60.0	1	1	32,228.00	NA	1	М	NA	ICU	PE
Ueki Y et al. ⁴² (2020)	Switzer- land	82.0	1	NA	10,000.00	NA	NA	М	NA	NA	PE, MI
Ullah W et al. ⁴³ (2020)	USA	59.0	1	1	1,280.00	NA	NA	F	LMWH	NA	PE
Xie Y et al. ⁴⁴ (2020)	China	57.0	2	NA	NA	NA	NA	2 M	NA	NA	PE
Zhou B et al. ⁴⁵ (2020)	China	69.0	1	1	8,000.00	NA	NA	М	NA	NA	AT, VT
Case series			0								
Beyrouti R et al. ⁴⁶ (2020)	UK	69.8	6	5	25,261.00	NA	NA	5 M, 1 F	NA	NA	IS
Bozzani A et al. ⁴⁷ (2020)	Italy	67.0	3	3	115,727.00	NA	NA	3 M	NA	NA	VT
Griffin DO et al. ⁴⁸ (2020)	USA	60.0	3	NA	NA	NA	NA	2 M, 1F	LMWH	NA	AT
Griffin DO et al. ⁴⁹ (2020)	USA	61.0*	3	3	12,597.00	NA	NA	3 M	NA	NA	PE
Oxley TJ et al. ⁵⁰ (2020)	USA	60.0	5	NA	NA	NA	NA	4M, 1 F	NA	NA	IS
Vulliamy P et al. ⁵¹ (2020)	UK	67.0	2	NA	NA	NA	NA	2 M	NA	NA	AT

*Median age

⁺LOS was reported as number of days

AMI: acute myocardial injury; AT: arterial thromboembolism; CS: cerebrovascular stroke; D: deceased; DI: discharge; DIS: disseminated intravascular disease; F: female; ICU; intensive care unit; IMV: intermittent mandatory ventilation; IS: ischaemic stroke; LOS: length of stay; LWMH: low-molecular weight heparin; M: male; MI: myocardial infarction; N: number of patients; NA: not applicable; PE: pulmonary embolism; T: thrombophlebitis; VT: venous thromboembolism.

The reported thromboembolic events were PE. thromboembolism, venous arterial thromboembolism, ischaemic stroke, myocardial infarction, and arterial thromboembolism. The most frequent thromboembolic event was PE, which was reported in 15 studies. Comorbidities were evaluated in 27 participants (48.21%). Ischaemic stroke was reported only in the studies of Oxley et al.⁵⁰ and Moshayedi et al.³⁹ The first study, included five patients admitted severe large-vessel stroke according with to National Institutes of Health Stroke Scale (NIHSS).⁵⁰ In the second study³⁹ the ischaemic stroke happened after coronary angioplasty and stent deployment, as the patient was admitted for

an ST-elevation myocardial infarction. Myocardial injury alone was reported only in two patients,^{39,42} both in their eighties.

Risk of Bias of the Included Studies

Confounding factors were unclear in observational studies as showed in risk of bias assessment.^{17,20,23,25} Questions related to confounding factors were not applicable to some observational studies.^{18,21,22,26-27,54} Adverse events questions did not apply to case reports.

Meta-Analysis Results

Fourteen studies were considered in the metaanalysis. The correlation among event rates is estimable only for moreancy and encompositions endpoints, reporting more than two pairwise event rates across studies. The correlation matrix among outcomes event rates is higher across PE and VTE outcomes, but it is not significant for all the pairwise comparisons. Some events were rare in the considered study populations; for example, the multi-variable pooled event rate of acute myocardial injuries was estimated to be 0.03 (95% CI: 0.00–0.07; p=0.23), while the meta-analytical estimate of disseminated intravascular disease is 0.04 (95% CI: 0.00-0.08; p=0.03) (Figure 2). Conversely, other events were found to be more frequent, the pooled proportion of pulmonary embolism was 0.14 (95% CI: 0.08-0.20; p<0.001), while the venous thromboembolism rate is 0.15 (95% CI: 0.09-0.30; p=0.04) (Figure 2). The pooled in-hospital mortality rate was equal to 0.12 (95% CI=0.08-0.16; p<0.001). The multivariable funnel plot does not reveal publication bias across reported evidence, only the study by Llitjos et al.¹⁸ was outside the triangle.

The multi-variable and univariate pooled estimates were similar especially for reported outcomes in more than six studies.

The I² heterogeneity levels, for all the reported outcomes (AMI, PE, VTE, disseminated intravascular disease, mortality), reveals a substantial heterogeneity with values exceeding the 60%. A significant modifying effect of the country (-0.081; 95% CI: -0.133, -0.029; p=0.0024) and study design (0.061; 95% CI: 0.007, 0.114; p=0.0274) has been evidenced. Moreover, mean age (0.009; 95% CI: -0.012, -0.005; p=<0.001) explains a considerable amount of heterogeneity by bringing I² values below the 75% threshold as indicated in the literature.⁵⁵

DISCUSSION

This meta-analysis showed that thromboembolism, particularly PE and VTE are frequent in patients hospitalised for COVID-19 with a pooled hospital mortality rate of 0.12. Coagulopathy is a common complication in patients with severe forms of COVID-19. In a recent study of 191 patients with COVID-19, 50% of in-hospital deaths were in individuals who had coagulopathy, compared to 7% of survivors. Of note, D-dimer levels >1,000 μ g/L were associated with death,⁵⁶ while IL-6 levels may correlate with

In a recent study on 183 patients, International Society on Thrombosis and Haemostasis (ISTH) criteria for disseminated intravascular coagulation (DIC) were met in 71% of patients who died.⁴ Some studies have reported that the incidence of DIC episodes were lower, ranging from 2% to 9% in patients admitted to ICU. Arachchillage et al.¹⁴ reported a higher incidence, probably because their patients had a great percentage of comorbidities 75 (41%) and higher levels of D-dimer (66,000 ng/mL).

SARS-CoV-2 may activate the coagulation system by using different pathogenetic mechanisms such as endothelial dysfunction, systemic inflammation, and a procoagulatory state; this may lead to pulmonary arterial thrombosis.⁵⁸⁻⁶⁰

Moreover, the clinical presentation of PE may overlap with that of COVID-19 pneumonia and may hinder the recognition of PE symptoms in patients who are already complaining of dyspnoea. Grillet et al.¹⁷ and Leonard-Lorant et al.²⁵ reported higher proportions of PE compared to other studies, likely because they only included patients who underwent CT angiogram. Poissy et al. compared the prevalence of PE in patients admitted in ICU in the same period of 2019 and 2020. Among patients in ICU with COVID-19 they reported 22 (20.6%) PE cases, which correspond to an absolute increase of 14.4% (95% CI; 6.1-22.8%) against control group of ICU patients admitted from February 27th to March 31st 2019.²¹

The authors suggest that actual estimates are more likely an underestimation of the real thrombosis prevalence in COVID-19. This has been confirmed by a recent autopsy study⁵⁴ where deep VTE was found in 58% of the cohort, and 30% of the patients had a PE as the direct cause of death. A recent metanalysis on 3,487 patients shown an incidence of VTE of 26% (95% prediction interval: 6, 66%) with an incidence of PE with or without DVT in 2% of patients (95% prediction interval: 2, 46%).⁵

Even in the studies where antithrombotic therapy (low-molecular weight heparin at prophylactic or therapeutic dosage) was routinely administered, the risk of thromboembolic complications seemed to remain considerable.



Figure 2: Forest plots of acute myocardial injury disseminated intravascular disease, venous thromboembolism, and pulmonary embolism. The random effect multi-variate pooled estimate has been reported together with the separate random effect meta-analysis pooled estimate with the 95% confidence interval.

This study confirms that in patients in ICU, the risk of thromboembolism is particularly high; therefore, use of therapeutic dose of antithrombotic drugs may be preferable. Despite this, the vast majority of the selected studies involved patients admitted to ICU, the incidence of VTE in patients with COVID-19 appeared to be higher than previously reported in critically ill patients with sepsis or septic shock.⁶¹ Llitjos et al.,¹⁸ in particular, reported a high prevalence of VTE (69%), which could be attributable to their performing of complex duplex ultrasound on admission as a standard of care in their ICU. In the studies with higher prevalence of VTE, except for Cui S et al.²² and Middeldorp S et al.,²⁰ part of the cohort was sustained by invasive mechanical ventilation.^{18,26}

Regarding myocardial events, the prevalence of myocardial lesions was found to be higher only in the study by Wei et al.²⁷ In fact, they reported an incidence of 33%, which is far above the 0.01% reported by Lodigiani et al.¹⁹ and Tavazzi et al.²⁶ This is may be related to the use of troponin

levels as the only diagnostic criteria for myocardial injury (14 pg/mL),²⁷ and this may overestimate the rate of true AMI due to embolic events, as differential diagnosis of elevated troponin levels includes nonspecific myocardial injury, impaired renal function, pericarditis, myocarditis, and shock, among others.

Limitations

This is a prevalence metanalysis: the study aim was to analyse prevalence of thromboembolism in patients affected by COVID-19. Predictors of thromboembolism events were not evaluated due to the lack of information about the characteristics of patients in the retrieved articles. For the same reason, the role of thromboembolism in affecting mortality risk was not assessed.

Although the authors chose to exclude case series and case reports from the meta-analysis, the results may have some limitations. The authors are not able to stratify the analysis according to pharmacological profile or characteristics of the patients due to the lack of information on anticoagulant therapy and characteristics of the patients. Further studies are needed to evaluate the impact of different antithrombotic treatments on thromboembolic events in this population. The design of the studies also reduced the quality of the results, as only one study had a form of group control.

CONCLUSIONS

This meta-analysis showed a high prevalence of thromboembolic events in patients

with COVID-19, in particular of VTE and PE. These findings may be of great clinical relevance, strongly suggesting to query PE in patients with COVID-19 when haemodynamic deterioration occurs, despite use of prophylactic antithrombotic treatments. These findings encourage the use of therapeutic dose of antithrombotic drugs in patients with COVID-19 in ICU. Further prospective studies are urgently needed to value the effect of prophylactic anticoagulant in such patients.

References

- Worldometer. COVID-19 Coronavirus Pandemic. Coronavirus Update (Live): 25,906,739 Cases and 861,289 Deaths from COVID-19 Virus Pandemic. 2020. Available at: https://www. worldometers.info/coronavirus/ Last accessed: April 15 2020.
- 2. Zaim S et al. COVID-19 and multiorgan re-sponse. Curr Probl Cardiol. 2020;45(8):100618.
- Chong PY et al. Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. Arch Pathol Lab Med. 2004;128(2):195-204.
- Tang N et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-7.
- Porfidia A et al. Venous thromboembolism in patients with COVID-19: systematic review and meta-analysis. Thromb Res. 2020;196:67-74.
- Lazzaroni MG et al. Coagulation dysfunction in COVID-19: the interplay between in-flammation, viral infection and the coagulation system. Blood Rev. 2021;46:100745.
- Liberati A et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009;339.
- Veritas Health Innovation. Covidence Systematic Review Software. Available at: www.covidence.org. Last accessed: 8 April 2021.
- Moola S et al, "Chapter 7: Systematic reviews of etiology and risk," Aromataris E, Munn Z (eds.), Joanna Briggs Institute Reviewer's Manual. 2020. The Joanna Briggs Institute. Available at: https://synthesismanual. jbi.global. Last accessed: 8 April 2021.

- Jackson D et al. Quantifying the impact of between-study heterogeneity in multi-variable metaanalyses. Stat Med. 2012;31(29):3805-20.
- The R Foundation. R Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.Rproject.org. Last accessed: 8 April 2021.
- Harrell FEJ. Regression Modeling Strategies. R package version 4.1-3. 2014. Available at: http://CRAN.Rproject.org/package=rms. Last accessed: 8 April 2021.
- Viechtbauer W. Conducting metaanalyses in R with the metafor package. Journal of Statistical Software. 2010;36(3):1-48.
- Arachchillage DRJ, Laffan M. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(5):1233-4.
- Cattaneo M et al. Pulmonary embolism or pulmonary thrombosis in COVID-19? Is the recommendation to use high-dose heparin for thromboprophylaxis justified? Thromb Haemost. 2020;120(8):1230-2.
- Chen T et al. Clinical characteristics of 113 deceased patients with coronavirus disease 2019: Retrospective study. The BMJ. 2020;368:m1091.
- Grillet F et al. Acute pulmonary embolism associated with COVID-19 pneumonia detected by pulmonary CT angiography. Radiol. 2020;296(3):e186-8.
- Llitjos JF et al. High incidence of venous thromboembolic events in anticoagulated severe COVID-19 patients. J Thromb Haemost. 2020;18(7):1743-6.

- Lodigiani C et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020;191:9-14.
- Middeldorp S et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18(8):1995-2002.
- 21. Poissy J et al. Pulmonary embolism in COVID-19 patients: awareness of an increased prevalence. Circulation. 2020;142:184-6.
- 22. Cui S et al. Prevalence of venous thromboembolism in patients with severe novel coronavirus pneumonia. Journal of Thrombosis and Haemostasis. 2020;18(6):1421-4.
- Helms J et al. High risk of thrombosis in patients with severe SARS-CoV-2 infection: a multicenter prospective cohort study. Intensive Care Med. 2020;1-10.
- 24. Klok FA et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-7.
- 25. Leonard-Lorant I et al. Acute pulmonary embolism in COVID-19 Patients on CT angiography and relationship to D-dimer levels. Radiol. 2020;296(3).
- 26. Tavazzi G et al. Thrombotic events in SARS-CoV-2 patients: an urgent call for ultrasound screening. Intensive Care Med. 2020;22:1-3.
- 27. Wei JF et al. Acute myocardial injury is common in patients with covid-19 and impairs their prognosis. Heart. 2020;106:1154-9.
- Audo A et al. Acute pulmonary embolism in SARS-CoV-2 infection treated with surgical embolectomy. The Annals of Thoracic Surgery. 2020;110(5):e403-4.

- 29. Cellina M, Oliva G. Acute pulmonary embolism in a patient with COVID-19 pneumonia. Diagn Interv Imaging. 2020;101(5):325-6.
- Danzi GB et al. Acute pulmonary embolism and COVID-19 pneumonia: a random association? Eur Heart J. 2020;41(19):1858.
- de Barry O et al. Arterial and venous abdominal thrombosis in a 79-year-old woman with COVID-19 pneumonia. Radiol Case Rep. 2020;15(7):1054-7.
- Dominguez-Erquicia P et al. Multivessel coronary thrombosis in a patient with COVID-19 pneumonia. Eur Heart J. 2020;41(22):2132.
- Fabre O et al. Severe acute proximal pulmonary embolism and COVID-19: a word of caution. The Ann Thorac Surg. 2020;110(5):e409-11.
- Foch E et al. Pulmonary embolism in returning traveler with COVID-19 pneumonia. J Travel Med. 2020;103(2):844-6.
- 35. Giacomelli E et al. Acute thrombosis of an aortic prosthetic graft in a patient with severe COVID-19 related pneumonia. Ann Vasc Surg. 2020:66:8-10.
- Harsch IA et al. SARS-CoV-2 pneumonia and pulmonary embolism in a 66-year-old female. Pol Arch Intern Med. 2020;130:438-9.
- Le Berre A et al. Concomitant acute aortic thrombosis and pulmonary embolism complicating COVID-19 pneumonia. Diagn Interv Imaging. 2020;101(5):321-2.
- Martinelli I et al. Pulmonary embolism in a young pregnant woman with COVID-19. Thromb Res. 2020;191:36-7.
- 39. Moshayedi P et al. Triage of acute ischemic stroke in confirmed COVID-19: large vessel occlusion associated with coronavirus infection. Front Neurol. 2020;11:353.
- 40. Rotzinger DC et al. Pulmonary embolism in patients with COVID-19:

time to change the paradigm of computed tomography. Thromb Res. 2020;190:58-9.

- Sulemane S et al. Acute pulmonary embolism in conjunction with intramural right ventricular thrombus in a SARS-CoV-2-positive patient. Eur Heart J Case Rep. 2021;5(1):ytaa388.
- 42. Ueki Y et al. ST-elevation myocardial infarction and pulmonary embolism in a patient with COVID-19 acute respiratory distress syndrome. Eur Heart J. 2020;41(22):2134.
- Ullah W et al. COVID-19 complicated by acute pulmonary embolism and right-sided heart failure. JACC Case Rep. 2020;2(9):1379-82.
- 44. Xie Y et al. COVID-19 complicated by acute pulmonary embolism. Radiol Cardiothorac Imaging. 2020;2(2):e200067.
- 45. Zhou B et al. Venous thrombosis and arteriosclerosis obliterans of lower extremities in a very severe patient with 2019 novel coronavirus disease: a case report. J Thromb Thrombolysis. 2020;50(1):229-32.
- Beyrouti R et al. Characteristics of ischaemic stroke associated with COVID-19. J Neurol Neurosurg Psychiatry. 2020;91(8):889-91.
- Bozzani A et al. SARS-CoV-2 infection and the upper limbs deep vein thrombosis risk. Ann Vasc Surg. 2020;66:11-3.
- Griffin DO et al. Arterial thromboembolic complications in COVID-19 in low-risk patients despite prophylaxis. Br J Haematol. 2020;190(1):e11-3.
- Griffin DO et al. Pulmonary embolism and increased levels of d-dimer in patients with coronavirus disease. Emerg Infect Dis. 2020;26(8):1941-3.
- 50. Oxley TJ et al. Large-vessel stroke as a presenting feature of Covid-19 in the young. NEJM. 2020;382:e60.
- 51. Vulliamy P et al. Acute aorto-iliac and mesenteric arterial thromboses as

presenting features of COVID-19. Br J Haematol. 2020;189(6):1053-4.

- 52. Yang F et al. Analysis of 92 deceased patients with COVID-19. J Med Virol. 2020;92(11):2511-5.
- 53. Magro C et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res. 2020;220:1-3.
- Wichmann D et al. Autopsy findings and venous thromboembolism in patients with COVID-19: a prospective cohort study. Ann Intern Med. 2020;173(4):268-77.
- Guyatt GH et al. GRADE guidelines:
 7. Rating the quality of evidenceinconsistency. J Clin Epidemiol. 2011;64(12):1294-302.
- 56. Zhou F et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet. 2020;395(10229):1054-62.
- 57. Libby P, Simon DI. Inflammation and thrombosis: the clot thickens. Circulation. 2001;103(13):1718-20.
- Giannis D et al. Coagulation disorders in coronavirus infected patients: COVID-19, SARS-CoV-1, MERS-CoV and lessons from the past. J Clin Virol. 2020;127:104362.
- 59. Li H et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. Lancet. 2020;395(10235):1517-20.
- Kluge S et al. German recommendations for critically ill patients with COVID 19. Med Klin Intensivmed Notfmed. 2020;115(3):175-7.
- Zusman O et al. Venous thromboembolism prophylaxis with anticoagulation in sep-tic patients: a prospective cohort study. QJM. 2015;108(3):197-204.

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New Therapeutic Approaches for Acute Myeloid Leukaemia

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Abstract

Acute myeloid leukaemia (AML) is a genetically heterogeneous haematopoietic neoplasm characterised by the accumulation of transformed immature blood progenitors in bone marrow. Since 1973, the backbone treatment has relied on the combination of cytarabine and an anthracycline, followed by allogeneic haematopoietic transplant if eligible. Therefore, the treatment decisions have largely revolved around chemotherapy drug intensity. Despite advances in our understanding of the underlying biology over the past decades, AML remains a therapeutic challenge as the overall survival is poor and treatment options are limited for relapsed/refractory AML or for unfit patients. After four decades without substantial changes, eight new noncytostatic drugs have been granted approval: vyxeos, enasidenib, gilteritinib, glasdegib, gemtuzumab ozogamicin, ivosidenib, midostaurin, and venetoclax. Despite promising preliminary results, some indications are based on early efficacy data, obtained in single-arm nonrandomised trials, highlighting the necessity for further validation in extended clinical trials. Interestingly, several druggable targets have been identified recently, associated with specific target-directed drugs. Based on the preclinical data available, great impact on clinical outcomes for patients with AML is expected, potentially increasing the therapeutic landscape for this disease.

ACUTE MYELOID LEUKAEMIA

Acute myeloid leukaemia (AML) is a lifethreatening, multifactorial haematological neoplasm characterised by the accumulation of transformed immature myeloid progenitors in bone marrow and peripheral haematopoietic organs. Multiple somatically acquired driver mutations, coexisting competing clones, and disease evolution over time account for the high heterogeneity observed in patients with AML, which results in a wide inter- and intrapatient variety of cellular phenotypes and clinical outcome.¹ Since the 1970s, the standard AML therapy consists of the '7+3' regimen, a combination of cytarabine and anthracycline.² This induction therapy aims to fully eliminate the leukaemic burden from the bloodstream and bone marrow. Once completed, an intensive chemotherapy cytarabine-based consolidation therapy, together with a haematopoietic cell transplantation if eligible, is followed to eliminate any potential, not necessarily detectable, leukaemic cells that may remain after induction.³ AML is mainly a disease of the elderly, with a median age at diagnosis of 68 years,⁴ which limits the treatment options due to comorbidities. The majority of patients will be considered unfit for intensive chemotherapy and will receive as treatment options lowdose cytarabine or hypomethylating agents (HMA), which generally fail to induce durable responses.⁵ Consequently, new treatment options are urgently needed for AML patients, especially for those unfit.

After decades without significant changes in treatment options, the pharmacologic landscape has unprecedentedly reshaped in the last 3 years with eight new drugs approved by regulatory agencies: vyxeos (CPX-351), enasidenib (AG221), gilteritinib (ASP2215), glasdegib (PF-04449913), gemtuzumab ozogamicin (GO), ivosidenib (AG120), midostaurin (PKC412), and venetoclax (ABT-199). However, only four drug approvals, CPX-351, ASP2215, GO, and PKC412, were based on randomised data with limitations in follow-up data availability. Optimisation of the schedule and treatment regimens, determination of safety, and potential synergistic effect with chemotherapy are still under evaluation. Nevertheless, several druggable targets have been described recently, associated with promising preclinical data that will likely have a great impact in clinical outcomes for patients with AML in the future. This review provides an overview of the novel AML treatment landscape and the most promising drugs under preclinical development that are expected to remarkably influence management of patients with AML.

FMS-RELATED TYROSINE KINASE 3

FMS-related tyrosine kinase 3 (FLT3) is one of the most frequent mutations in AML, representing approximately 30% of mutations in patients with AML,⁶ and are considered drivers in the majority of the cases. *FLT3* mutations can be found either as internal tandem duplications in the juxtamembrane domain (*FLT3*-ITD;

20%) or point mutations in the tyrosinekinase domain (*FLT3*-TKD; 10%). *FLT3* plays a key role in controlling survival, differentiation, and proliferation of haematopoietic cells, and both *FLT3*-ITD and *FLT3*-TK constitutively activate its kinase activity. While *FLT3*-ITD has prognostic value,⁷ *FLT3*-TK is weakly associated with clinical outcome.⁸

First-generation FLT3 inhibitors, such as PKC412, are promiscuous, inhibiting several tyrosine kinases, with a transient pharmacological effect, especially when used in monotherapy in relapsed AML. This lack of specificity and high affinity may contribute to adverse effects from inhibition of multiple other kinases.9 Although it initially demonstrated only modest single-agent activity, improved response rate and survival were achieved with PKC412 in combination with chemotherapy (complete response [CR]: 59% versus 54% in the placebo group),¹⁰ which supported its approval by the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) in 2017. PKC412 is indicated for FLT3 mutation-positive AML in combination with standard induction and consolidation chemotherapy. Secondgeneration FLT3 inhibitors, including ASP2215,11 crenolanib,¹² and AC220 (quizartinib),¹³ display higher potency and specificity for FLT3, with a more restricted tyrosine-kinase recognition profile. ASP2215, a dual FLT3/AXL inhibitor, has been shown to be effective in a singleagent regimen in relapsed/refractory AML with FLT3 mutation (CR: 14% versus 11% in the salvage chemotherapy control group), inducing manageable side effects,¹⁴ leading to its recent approval by the FDA in 2018 and EMA in 2019 as a monotherapy in FLT3 mutation-positive relapsed/refractory AML. However, extensive randomised clinical trials are needed for these drugs to properly define the AML patient population suitable for each FLT3 inhibitor approved, as approvals for PKC412 and ASP2215 were controversial due to the marginal improvement reported.¹⁵ Based on their broad inhibition targets besides FLT3, these inhibitors may also improve the prognosis in AML without FLT3 mutations.

According to the mode of binding, *FLT3* inhibitors can be further divided into Type I and II. Type I *FLT3* inhibitors target the ATP binding site of the active conformation and

are effective against *FLT3*-ITD and *FLT3*-TKD mutations. However, Type II inhibitors recognise a pocket adjacent to the ATP binding site that is accessible in the inactive conformation: only preventing the activity of ITD mutations but sparing TKD mutations. Both PKC412 and ASP2215 are Type I inhibitors, whereas AC220 belongs to the Type II group.¹⁶ In concordance, both PKC412 and ASP2215 promoted a consistent improvement in overall survival in all *FLT3* mutation subtypes.^{10,17} The Type II inhibitor AC220 does not have significant activity against *FLT3*-TKD mutations,¹⁸ which is an established mechanism of resistance in AC220-treated patients.¹⁹

Despite significant progress in the development of better *FLT3* inhibitors, emergence of resistances constitutes a major challenge.²⁰ Resistance mechanisms include acquisition of secondary-driven mutations²¹ and modulation of alternative signalling pathways.²² Several next-generation *FLT3* inhibitors are being preand clinically evaluated.

ISOCITRATE DEHYDROGENASE

The isocitrate dehydrogenases (IDH) are key enzymes implicated in metabolism, located in cytoplasm (IDH1) and mitochondria (IDH2). Mutations in IDH1 and IDH2 genes are found in 10% of patients with AML.²³ IDH-based targeted therapies include small molecules aiming to restore normal IDH function and block the production or downstream effects of intermediate metabolites. IDH1-specific AG120 and IDH2-specific AG221 are potent in vivo mutant IDH inhibitors with high treatment responses in clinical trials (AG120 CR: 29%; AG221 CR: 19%), although no control arm was included.^{24,25} AG221 was approved by the FDA in 2017 for IDH2 mutation-positive relapsed/ refractory AML. Similarly, the FDA also approved AG120 for IDH1-mutant relapsed/ refractory AML (2018) and newly diagnosed (age ≥75 years) IDH1-mutant AML noneligible for intensive induction chemotherapy (2019). However, neither has been approved by the EMA. Mutant-IDH inhibition prompts differentiation of leukaemic cells and the emergence of functional neutrophils, suggesting that efficiency is based on differentiation induction.^{24,25} A constitutive activation of the

RAS/MAPK pathway was associated with low responders in AG221-treated patients and warrants further investigation and analysis of rational combinations in order to overcome this resistance.²⁶ However, the use of IDH inhibitors may lead to the IDH differentiation syndrome, which results in the release of inflammatory cytokines, constituting a serious safety concern.²⁷ Improvement in the pharmacokinetic (avoiding rapid metabolism and clearance) and pharmacodynamic (increasing the specificity for mutant IDH, reducing undesirable effects, and increasing the therapeutic window) profiles are necessary to optimise the therapeutic potential for AML; new small molecules are under preclinical and clinical evaluation.

B-CELL LYMPHOMA 2

The B-cell lymphoma 2 (Bcl-2) family members tightly regulate programmed cell death and are divided in three different subfamilies based on their functionality: antiapoptotic multidomain proteins (i.e., Bcl-2), proapoptotic multidomain proteins (i.e., Bax), and proapoptotic BH3only proteins (i.e., BID). Bcl-2 is overexpressed in AML cells and its expression is associated with chemoresistance.^{28,29} Despite promising results in preclinical models,³⁰ the efficacy in monotherapy of the first clinical-grade Bcl-2-specific inhibitor, ABT-199, is limited.³¹ Previously, obatoclax, a BH3 mimetic and pan-Bcl-2 inhibitor, also showed minimal activity as a single agent in patients with AML, in contrast to its potent antileukaemic effect in preclinical settings.³² However, ABT-199 acts synergically with HMA to induce mitochondriamediated apoptosis in AML cells (venetoclaxazacytidine CR: 37%; venetoclax-decitabine CR: 54%; no control arm was present in these studies), probably due to the HMA-mediated downregulation of McI-1, in which overexpression confers resistance to ABT-199.^{33,34} Alternatively, the durable remission observed in combination treatment (12.5 months, no control arm was present in the study) might be due to metabolic perturbations in the most primitive leukaemic cells.³⁵ ABT-199 was granted approval (FDA 2018) for elderly (\geq 75 years) unfit patients with AML in combination with HMA, although a confirmatory Phase III clinical trial, performed as a randomised, placebo-controlled study,

will be required to definitively measure its clinical benefit. Nevertheless, resistance to ABT-199 appears in 30% of patients with AML and neither the mechanism responsible nor a biomarker have been identified.³³

HEDGEHOG

The hedgehog (Hh) signalling pathway plays a key role in the development and homeostasis of many organs and tissues, and its aberrant activation is associated with tumourigenesis.³⁶ The importance of Hh in AML is controversial as no direct evidence of an aberrant regulation of Hh in AML cells has been consistently found.³⁷⁻³⁹ However, the development of PF-04449913, a clinical-grade smoothened (the receptor responsible for the signalling transduction) inhibitor, demonstrated that inhibition of the Hh signalling pathway severely impaired the most primitive leukaemic cell functionality and restored chemosensitivity.40 However, the antileukaemic effect was at least partially mediated by nonhaematopoietic stroma cells, as previously demonstrated.³⁷ While the clinical efficacy was evident in combination with lowdose cytarabine (CR: 17% versus 2% in the control arm), the overall response rate remained modest (overall survival: 8.8 months versus 4.9 months in the control arm), suggesting that optimisation in treatment regimens are still needed.⁴¹ Nevertheless, PF-04449913 was approved by the FDA in 2018 for untreated elderly (≥75 years) unfit patients with AML in combination with HMA.

OLD CONCEPTS, NEW DRUGS, AND INDICATIONS

The backbone of the first-line induction therapy is composed of the combination of cytarabine and an anthracycline (either daunorubicin or idarubicin).² Based on the synergism associated with the coadministration of cytarabine and daunorubicin in a specific molar ratio (from 1:1 to 10:1),⁴² the liposomal formulation CPX-351 containing a fixed, synergy-proven 5:1 ratio of cytarabine and daunorubicin was developed. This encapsulation of the conventional chemotherapeutics prolongs the half-life and increases the bioavailability, as compared to the free drugs, enhancing the antileukaemic effect and inducing similar side effects.^{42,43} Surprisingly, CPX-351 improved response rates in unfit and relapsed high-risk AML compared to free drugs (CR: 37% versus 26% in the control arm), without significant clinical benefit in favourable- and intermediate-risk groups.⁴⁴⁻⁴⁶ CPX-351 constitutes mainly an improved version of the conventional combination of free cytarabine plus daunorubicin; as such, a quantitative benefit is expected rather than a qualitative one. CPX-351 has recently been approved for newly diagnosed, therapyrelated AML and AML with myelodysplasiarelated changes, by both the FDA (2017) and EMA (2018).

GO (mylotarg[™]♥, Pfizer, New York, New York, USA) is a humanised anti-CD33 monoclonal antibody conjugated to the cytotoxic agent calicheamicin. CD33 is a panmyeloid marker expressed in most AML cells and healthy myeloid progenitors and mature cells. In 2000, GO received approval for CD33-positive AML in first remission not eligible for induction therapy.⁴⁷ The confirmatory study failed to demonstrate clinical benefit for GO and demonstrated a higher rate of severe adverse effects.⁴⁸ In 2010, GO was withdrawn voluntarily from the market. However, several clinical trials have recently shown a clinical benefit in newly diagnosed patients with CD33-positive AML (CR: 73% versus 72% in the control arm), although no evidence of benefit for adverseand intermediate-risk patients has been described.⁴⁹ GO is currently approved for newly diagnosed CD33-positive AML (FDA 2017, EMA 2018) and CD33-positive relapsed/refractory AML (FDA 2017).

IMMUNOTHERAPY-BASED TREATMENTS IN ACUTE MYELOID LEUKAEMIA

In the last decade, major efforts have been made to develop the adoptive transfer of immune cells (T or NK cells) expressing a genetically engineered chimeric antigen receptor (CAR) in AML, following the success of this approach in CD19+ B-cell acute lymphoblastic leukaemia (B-ALL).⁵⁰ Despite numerous preclinical studies targeting different antigens (i.e., CD123, CD33, FLT3, CLL-1, and LeY), relatively few CAR-based approaches have been investigated in clinical trials in AML, showing a delay in the disease progression without achieving remission.⁵¹ Although the first clinical-grade CAR T in AML was generated against LeY,52 CD33 and CD123 are currently more attractive targets, especially CD123 because of its high expression on the most primitive AML cell fraction⁵³ and its association with poor clinical outcome.⁵⁴ Both antigens are almost ubiquitous in AML cells, and in several normal haematopoietic cell subpopulations.^{55,56} To date, the fundamental biological barrier limiting the application of CAR T cell therapy to AML is the absence of truly AML-specific surface antigens.⁵⁶⁻⁵⁸ The prolonged myeloablation resulting from CAR T cells targeting healthy myeloid and AML cells is ultimately fatal, similar to other 'ontarget off-tumour' toxicities. Additionally, microenvironment the AML is highly immunosuppressive, impacting the efficacy of immune-related therapies. The potent myeloablation induced by AML CAR T cells could serve as a novel conditioning regimen prior to allogeneic transplantation.⁵⁹

Several immune inhibitory ligands and their receptors have been extensively studied in cancer as potential modulators of key immune checkpoints. Despite the success in solid tumours, blocking monoclonal antibodies against PD-1 (nivolumab and pembrolizumab) and CTLA-4 (ipilimumab) have provided limited results in AML.^{60,61} Combination of immune checkpoint inhibitors with HMA could represent an interesting strategy in AML.⁶² Other immune-related approaches based on antibody-drug conjugates, blocking antibodies, and dual-affinity retargeting (DART) proteins are under evaluation in early-phase trials.

NEUROTRANSMITTER RECEPTORS

Classic neurotransmitter receptors have from attracted increasing attention investigators in oncology in recent years. Both dopamine and serotonin receptors are highly expressed in AML and serve as prognostic biomarkers and therapeutic targets as their pharmacological inhibition impairs AML viability in relevant preclinical models.^{63,64} Furthermore, the initial results in clinical trials indicated that a promiscuous dopamine D2 receptor antagonist reduced the leukaemia burden, although several off-target side effects discourage

its use.⁶⁵ Interestingly, inhibition of both neurotransmitter receptors induced terminal differentiation of AML cells, greatly affecting the most primitive leukaemic cell population. As the block in differentiation is a common feature of all AML subtypes and differentiated cells are more sensitive to chemotherapy and lose any self-renewal capacity, differentiationbased therapies are very attractive approaches. Based on the promising preclinical data,^{63,64} the differential expression of both receptors enable the existence of a feasible therapeutic window to maximise the pharmacological antileukaemic effects without major side effects, supporting the further preclinical and clinical development of specific dopamine and serotonin receptor antagonists for AML.

ORGANELLE-DIRECTED APPROACHES

Similar to solid tumour cells, AML cells present several alterations in cell organelles. At a mitochondrial level, a distinct glucose metabolism signature,⁶⁶ an increased reactive oxygen species content,⁶⁷ greater copy numbers of mitochondrial DNA,68 and electron transportation chain dysregulation⁶⁹ are common features in AML cells to accommodate all the neoplastic requirements. Drugging mitochondrial oxidative phosphorylation (OXPHOS) using different direct and indirect approaches demonstrated promising preclinical specific antileukaemic results,⁷⁰⁻⁷³ especially considering that chemotherapy-resistant AML cells are highly dependent on high oxidative phosphorylation status.⁷⁴

Similarly, the augmented metabolic demands associated with the transformation events result in the upregulation of the lysosomal function in leukaemic cells, inducing an increase in lysosomal biogenesis and mass together with the activation of cathepsins⁷⁵ and changes in the sphingolipid metabolism.⁷⁶ As a consequence of these lysosomal changes, AML cells present fragile lysosomes because of destabilisation of the lysosomal limiting membrane and lower pH. Currently, there is a growing interest in a group of structurally diverse small molecules classified as cationic amphiphilic drugs (CAD), defined by a hydrophobic ring moiety and an amine group.⁷⁷ Due to their physicochemical properties, CAD passively diffuse through

biological membranes and a process known as ion trapping enables their accumulation in lysosomes, eventually inducing a lysosomedependent programmed cell death.^{75,78,79} Preclinical data suggest that CAD are highly specific and widely safe; however, their pharmacological profiles are suboptimal and drug delivery improvement might be required for clinical development of this drug family.

EPIGENETIC MODULATORS

Despite its high heterogeneity, a low mutation burden is found in AML.⁸⁰ However, global alterations in DNA methylation patterns are complex, further supporting the notion that epigenetic heterogeneity better explains leukaemia identity compared with the genetic background.⁸¹⁻⁸³ Thus, the AML epigenome has emerged as a new and exciting target for drug discovery.

The histone demethylase LSD-1 plays key roles during oncogenesis, becoming an emerging target for haematological and solid tumours. Several LSD-1 inhibitors are under clinical assessment for AML, such as ORY-1001⁸⁴ and GSK-2879552.⁸⁵ Despite the promising data generated in mouse models, these LSD-1 targeted agents induced unsubstantial overall response rates in patients with relapsed/

refractory AML.⁸⁶ As the preclinical data suggested the possibility of synergistic effects of LSD-1 with already approved HMA,⁸⁴ clinical trials to evaluate the combination therapy are ongoing; therefore, the future clinical development of LSD-1 inhibitors for AML therapy is uncertain.

FUTURE PERSPECTIVE

While advances in supportive care and prognostic risk stratification have optimised the performance of standard chemotherapy, overall long-term survival remains poor. Moreover, for four decades the treatment paradigm has been based on a simple binary distinction: intensive chemotherapy (potentially curative) and low-intensity regimens (palliative). Since 2017, several new drugs have been approved, incorporating the notion of personalised treatments for AML. However, caution should be highlighted until more extensive, carefully designed clinical trial studies are terminated and analysed. Considering the preliminary data already available, novel targeted therapies based on new mechanisms of action are likely to be more successful than current treatments, as effective antileukaemic activity with reduced toxicity is expected. In any case, validation in clinical trials should be conducted.

References

- Arber DA et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391-405.
- Yates JW et al. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. Cancer Chemother Rep. 1973;57(4):485-8.
- Döhner H et al. Acute myeloid leukemia. N Engl J Med. 2015:373:1136-52.
- Noone A et al. Acute myeloid leukemia. Cancer Stat Facts. 2018. Available at: https://seer.cancer. gov/statfacts/html/amyl.html. Last accessed: 14 August 2020.
- Ossenkoppele G, Löwenberg B. How I treat the older patient with acute myeloid leukemia. Blood. 2015;125(5):767-74.

- Schnittger S et al. Analysis of *FLT3* length mutations in 1003 patients with acute myeloid leukemia: correlation to cytogenetics, FAB subtype, and prognosis in the AMLCG study and usefulness as a marker for the detection of minimal residual disease. Blood. 2002;100(1):59-66.
- Port M et al. Prognostic significance of *FLT3* internal tandem duplication, nucleophosmin 1, and *CEBPA* gene mutations for acute myeloid leukemia patients with normal karyotype and younger than 60 years: a systematic review and meta-analysis. Ann Hematol. 2014;93(8):1279-86.
- Bacher U et al. Prognostic relevance of *FLT3*-TKD mutations in AML: the combination matters—an analysis of 3082 patients. Blood. 2008;111(5):2527-37.
- 9. Propper DJ et al. Phase I and pharmacokinetic study of PKC412, an inhibitor of protein kinase c. J Clin

Oncol. 2001;19(5):1485-92.

- Stone RM et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a *FLT3* mutation. N Engl J Med. 2017;377:454-64.
- Kampa-Schittenhelm KM et al. Quizartinib (AC220) is a potent second generation Class III tyrosine kinase inhibitor that displays a distinct inhibition profile against mutant-*FLT3*, *-PDGFRA* and *-KIT* isoforms. Mol Cancer. 2013;12:19.
- Kampa-Schittenhelm KM et al. Crenolanib is a Type I tyrosine kinase inhibitor that inhibits mutant *KIT D816* isoforms prevalent in systemic mastocytosis and core binding factor leukemia. Oncotarget. 2017;8:82897-909.
- Cortes JE et al. Phase I study of quizartinib administered daily to patients with relapsed or refractory acute myeloid leukemia irrespective

of FMS-like tyrosine kinase 3-internal tandem duplication status. J Clin Oncol. 2013;31(29):3681-867.

- Perl AE et al. Selective inhibition of *FLT3* by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, openlabel, Phase 1-2 study. Lancet Oncol. 2017;18(8):1061.
- Estey E et al. Recent drug approvals for newly diagnosed acute myeloid leukemia: gifts or a Trojan horse? Leukemia. 2020;34(3):671-81.
- Liu Y, Gray NS. Rational design of inhibitors that bind to inactive kinase conformations. Nat Chem Biol. 2006;2(7):358-64.
- Perl AE et al. Gilteritinib or chemotherapy for relapsed or refractory *FLT3*-mutated AML. N Engl J Med. 2019;381:1728-40.
- Smith CC et al. *FLT3 D835* mutations confer differential resistance to Type II *FLT3* inhibitors. Leukemia. 2015;29:2390-2.
- Smith CC et al. Validation of ITD mutations in *FLT3* as a therapeutic target in human acute myeloid leukaemia. Nature. 2012;485(7397):260-3.
- 20. Daver N et al. Secondary mutations as mediators of resistance to targeted therapy in leukemia. Blood. 2015;125(21):3236-45.
- von Bubnoff N et al. FMS-like tyrosine kinase 3-internal tandem duplication tyrosine kinase inhibitors display a nonoverlapping profile of resistance mutations in vitro. Cancer Res. 2009;69:3032-41.
- 22. Piloto O et al. Prolonged exposure to *FLT3* inhibitors leads to resistance via activation of parallel signaling pathways. Blood. 2007;109(4):1643-52.
- Mardis ER et al. Recurring mutations found by sequencing an acute myeloid leukemia Genome. N Engl J Med. 2009;361:1058-66.
- Stein EM et al. Enasidenib in mutant *IDH2* relapsed or refractory acute myeloid leukemia. Blood. 2017;130(6):722-31.
- DiNardo CD et al. Durable remissions with ivosidenib in *IDH1*-mutated relapsed or refractory AML. N Engl J Med. 2018;378:2386-98.
- Amatangelo MD et al. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. Blood. 2017;130(6):732-41.
- 27. DiNardo CD et al. Novel therapeutics in acute myeloid leukemia. Am Soc Clin Oncol Educ B. 2017;37:495-503.
- Campos L et al. High expression of bcl-2 protein in acute myeloid leukemia cells is associated with poor response to chemotherapy. Blood. 1993;81(11):3091-6.

- 29. Moon JH et al. *BCL2* gene polymorphism could predict the treatment outcomes in acute myeloid leukemia patients. Leuk Res. 2010;34(2):166-72.
- Pan R et al. Selective BCL-2 inhibition by ABT-199 causes on-target cell death in acute myeloid leukemia. Cancer Discov. 2014;4(3):362-75.
- Konopleva M et al. Efficacy and biological correlates of response in a Phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. Cancer Discov. 2016;6(10):1106-17.
- 32. Schimmer AD et al. A multicenter Phase I/II study of obatoclax mesylate administered as a 3- or 24-hour infusion in older patients with previously untreated acute myeloid leukemia. PLoS One. 2014;9(10):e108694.
- DiNardo CD et al. Venetoclax combined with decitabine or azacitidine in treatment-naive, elderly patients with acute myeloid leukemia. Blood. 2019;133(1):7-17.
- 34. Pollyea DA et al. Venetoclax in combination with hypomethylating agents induces rapid, deep, and durable responses in patients with aml ineligible for intensive therapy. Blood. 2018;132:285.
- Pollyea DA et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. Nat Med. 2018;24(12):1859-66.
- Amakye D et al. Unraveling the therapeutic potential of the Hedgehog pathway in cancer. Nat Med. 2013;19(11):1410-22.
- 37. Boyd AL et al. Nonhematopoietic cells represent a more rational target of *in vivo* hedgehog signaling affecting normal or acute myeloid leukemia progenitors. Exp Hematol. 2013;41:858-69.e4.
- Hofmann I et al. Hedgehog signaling is dispensable for adult murine hematopoietic stem cell function and hematopoiesis. Cell Stem Cell. 2009;4(6):559-67.
- 39. Kobune M et al. Drug resistance is dramatically restored by hedgehog inhibitors in CD34+ leukemic cells. Cancer Sci. 2009;100:948-55.
- Fukushima N et al. Small-molecule hedgehog inhibitor attenuates the leukemia-initiation potential of acute myeloid leukemia cells. Cancer Sci. 2016;107(10):1422-9.
- Cortes JE et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. Leukemia. 2019;33(2):379-89.
- 42. Tardi P et al. In vivo maintenance of

synergistic cytarabine: daunorubicin ratios greatly enhances therapeutic efficacy. Leuk Res. 2009;33(1):129-39.

- 43. Feldman EJ et al. First-in-man study of CPX-351: a liposomal carrier containing cytarabine and daunorubicin in a fixed 5:1 molar ratio for the treatment of relapsed and refractory acute myeloid leukemia. J Clin Oncol. 2011;29(8):979-85.
- 44. Lancet JE et al. CPX-351 (cytarabine and daunorubicin) liposome for injection versus conventional cytarabine plus daunorubicin in older patients with newly diagnosed secondary acute myeloid leukemia. J Clin Oncol. 2018;36:2684-92.
- 45. Lancet JE et al. Phase 2 trial of CPX-351, a fixed 5:1 molar ratio of cytarabine/ daunorubicin, vs cytarabine/daunorubicin in older adults with untreated AML. Blood. 2014;123(21):3239-46.
- 46. Cortes JE et al. Phase II, multicenter, randomized trial of CPX-351 (cytarabine:daunorubicin) liposome injection versus intensive salvage therapy in adults with first relapse AML. Cancer. 2015;121(2):234-42.
- 47. Bross PF et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. Clin Cancer Res. 2001;7(6):1490-6.
- Petersdorf SH et al. A Phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. Blood. 2013;121(24):4854-60.
- 49. Castaigne S et al. Effect of gemtuzumab ozogamicin on survival of adult patients with *de-novo* acute myeloid leukaemia (ALFA-0701): a randomised, open-label, Phase 3 study. Lancet. 2012;379(9825):1508-16.
- 50. Maude SL et al. Chimeric antigen receptor t cells for sustained remissions in leukemia. N Engl J Med. 2014;371:1507-17.
- 51. Cummins KD, Gill S. Chimeric antigen receptor T-cell therapy for acute myeloid leukemia: how close to reality? Haematologica. 2019;104:1302-8.
- 52. Ritchie DS et al. Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia. Mol Ther. 2013;21:2122-9.
- 53. Jordan CT et al. The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stems cells. Leukemia. 2000;14(10):1777-84.
- Testa U et al. Elevated expression of IL-3Rα in acute myelogenous leukemia is associated with enhanced blast proliferation, increased cellularity, and poor prognosis. Blood. 2002;100(8):2980-8.

- 55. Testa U et al. CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. Biomark Res. 2014;2(1):4.
- Wang QS et al. Treatment of CD33directed chimeric antigen receptormodified T cells in one patient with relapsed and refractory acute myeloid leukemia. Mol Ther. 2015;23(1):184-91.
- Luo Y et al. First-in-man CD123specific chimeric antigen receptormodified T cells for the treatment of refractory acute myeloid leukemia. Blood. 2015;126(23):3778.
- Cummins KD et al. Treating relapsed / refractory (RR) AML with biodegradable anti-CD123 CAR modified T cells. Blood. 2017;130:1359.
- 59. Liu F et al. First-in-Human CLL1-CD33 compound CAR T cell therapy induces complete remission in patients with refractory acute myeloid leukemia: update on Phase 1 clinical trial. Blood. 2018;132:901.
- 60. Daver N et al. Phase IB/II study of nivolumab in combination with azacytidine (AZA) in patients (pts) with relapsed acute myeloid leukemia (AML). Blood. 2016;128(22):763.
- Daver NG et al. Azacitidine (AZA) with nivolumab (Nivo), and AZA with nivo + ipilimumab (Ipi) in relapsed/ refractory acute myeloid leukemia: a non-randomized, prospective, Phase 2 study. Blood. 2019;134(Suppl 1):830.
- Daver N et al. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/ refractory acute myeloid leukemia: a nonrandomized, open-label, Phase II study. Cancer Discov. 2019;9(3):370-83.
- Sachlos E et al. Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells. Cell. 2012;149(6):1284-97.
- 64. Etxabe A et al. Inhibition of serotonin receptor Type 1 in acute myeloid leukemia impairs leukemia stem cell functionality: a promising novel therapeutic target. Leukemia. 2017;31(11):2288-302.

- 65. Aslostovar L et al. A Phase 1 trial evaluating thioridazine in combination with cytarabine in patients with acute myeloid leukemia. Blood Adv. 2018;2(15):1935-45.
- 66. Chen WL et al. A distinct glucose metabolism signature of acute myeloid leukemia with prognostic value. Blood. 2014;124(10):1645-54.
- 67. Hole PS et al. Overproduction of NOX-derived ROS in AML promotes proliferation and is associated with defective oxidative stress signaling. Blood. 2013;122(19):3322-30.
- Škrtić M et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. Cancer Cell. 2011;20(5):674-88.
- 69. Sriskanthadevan S et al. AML cells have low spare reserve capacity in their respiratory chain that renders them susceptible to oxidative metabolic stress. Blood. 2015;125(13):2120-30.
- Baccelli I et al. Mubritinib targets the electron transport chain complex I and reveals the landscape of oxphos dependency in acute myeloid leukemia. Cancer Cell. 2019;36:84-99.
- Lagadinou ED et al. BCL-2 inhibition targets oxidative phosphorylation and selectively eradicates quiescent human leukemia stem cells. Cell Stem Cell. 2013;12(3):329-41.
- 72. Pei S et al. AMPK/FIS1-mediated mitophagy is required for self-renewal of human aml stem cells. Cell Stem Cell. 2018;23:86-100.
- Molina JR et al. An inhibitor of oxidative phosphorylation exploits cancer vulnerability. Nat Med. 2018;24(7):1036-46.
- 74. Farge T et al. Chemotherapyresistant human acute myeloid leukemia cells are not enriched for leukemic stem cells but require oxidative metabolism. Cancer Discov. 2017;7(7):716-735.
- 75. Sukhai MA et al. Lysosomal disruption preferentially targets acute myeloid leukemia cells and progenitors. J Clin

Invest. 2013;123(1):315-28.

- Kitatani K et al. Role of sphingolipids and metabolizing enzymes in hematological malignancies. Mol Cells. 2015;38(6):482-95.
- Kornhuber J et al. Identification of new functional inhibitors of acid sphingomyelinase using a structureproperty-activity relation model. J Med Chem. 2008;51(2):219-37.
- 78. Cornet-Masana JM et al. Dual lysosomal-mitochondrial targeting by antihistamines to eradicate leukaemic cells. EBioMedicine. 2019;47:221-34.
- Nielsen IØ et al. Cationic amphiphilic drugs induce lipidome-wide changes and cell death in leukemia cells. Metabolomics. 2020;16(9).
- Eisfeld AK et al. The mutational oncoprint of recurrent cytogenetic abnormalities in adult patients with *de novo* acute myeloid leukemia. Leukemia. 2017;31(10):2211-8.
- 81. Figueroa ME et al. DNA methylation signatures identify biologically distinct subtypes in acute myeloid leukemia. Cancer Cell. 2020;17(1):13-27.
- Corces MR et al. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nat Genet. 2016;48(10):1193-203.
- Li S et al. Distinct evolution and dynamics of epigenetic and genetic heterogeneity in acute myeloid leukemia. Nat Med. 2016;22(7):792-9.
- Maes T et al. ORY-1001, a potent and selective covalent *KDM1A* inhibitor, for the treatment of acute leukemia. Cancer Cell. 2018;33:495-511.
- 85. Smitheman KN et al. Lysine specific demethylase 1 inactivation enhances differentiation and promotes cytotoxic response when combined with all-trans retinoic acid in acute myeloid leukemia across subtypes. Haematologica. 2019;104(6):1156-67.
- Fang Y et al. LSD1/KDM1A inhibitors in clinical trials: advances and prospects. J Hematol Oncol. 2019;12(1):129.

Improvements and Shortcomings in Emergency Oxygen Prescribing: A Quality Improvement Initiative at an Acute Tertiary Care Hospital

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Abstract

Oxygen is one of the most commonly used yet poorly prescribed drugs. The 2015 British Thoracic Society (BTS) emergency oxygen audit highlighted the national shortcomings in oxygen prescribing and administration. A 2017 local audit at the Royal Sussex County Hospital, Brighton, UK, continued to demonstrate poor compliance with the BTS Oxygen Prescribing Guidelines in all areas audited. This study carried out yearly reaudits in November 2018 and 2019 to objectively measure the impact of implementing trust-wide and local interventions (July 2018 and August 2019).

Intervention 1 included introduction of the National Early Warning Score (NEWS2) scale and redesigning drug charts with tick-boxes for target oxygen saturations. Intervention 2 included mandatory junior doctor teaching on safe oxygen prescribing, 'oxygen safety' posters on audited wards, and reminders at handover for staff to measure and document oxygen saturations.

Following Intervention 1, all patients with valid oxygen prescriptions had a specified target saturations range. Intervention 2 ensured all patients had actual saturations within their prescribed target range, and 99% had oxygen saturations documented with sufficient frequency for their NEWS2 score. These were huge improvements from previous audits, during which a significant proportion of patients were at risk of hypercapnia, and those over- or underoxygenated were left unrecognised for hours. Despite improvements, 14% of patients continued to use oxygen without valid prescriptions in 2019, and drug charts were inconsistently signed for during drug rounds.

Although the implemented changes enabled drastic improvements for patient safety and quality in oxygen use, future work should ensure oxygen is always treated as a drug with suitable prescription and documentation.

BACKGROUND

There have been seven national audits looking at the prescription of oxygen, the largest of which

was the 2015 British Thoracic Society (BTS) Audit, which showed shortcomings in oxygen prescribing practices. This subsequently led to the publication of the Emergency Oxygen Prescribing Guidelines and set the National Improvement Objectives.

2017 audit at acute tertiary Α an continued highlight care hospital to а failure to meet the expected standards. A reaudit was carried out to determine whether the implementation of trust-wide changes in July 2018, namely the introduction of a National Early Warning Score 2 (NEWS2) and newly designed drug charts, had impacted these behaviours. This study carried out yearly reaudits in November 2018 and 2019 to objectively measure the impact of implementing trustwide and local interventions (in July 2018 and August 2019). The 2018 and 2019 audits aimed to compare current oxygen prescribing and delivery practices at the hospital to standards set by the 2015 BTS National Improvement Objectives, and evaluate changes made since the 2017 audit. Adult inpatients on four wards were audited on a single occasion using a standardised questionnaire validated by the BTS.

INTRODUCTION

Oxygen is one of the most commonly used yet poorly prescribed drugs. There is a risk that patients may be given too little or too much oxygen, thereby increasing their mortality risk. Following the 2015 BTS National Emergency Oxygen Prescribing Audit and publication of the Emergency Oxygen Prescribing Guidelines, which showed national shortcomings,^{1,2} a 2017 audit at an acute tertiary care hospital continued to highlight a failure to meet the expected standards for oxygen prescribing and administration.

AIMS

То compare prescribing and oxygen delivery practices at an acute tertiary care hospital to standards set by the 2015 BTS National Improvement Objectives in yearly audits, and to evaluate improvements in oxygen prescribing from the changes that were implemented following a 2017 local oxygen prescribing audit. Improvements included introduction of the NEWS2 scale, new drug charts with a tick-box for target saturations ranges, educational initiatives for doctors, posters, and face-to-face reminders

on oxygen prescribing guidelines for nursing staff.

OBJECTIVES

Primary Objectives

Based on the 2015 BTS National Improvement Objectives,¹ the authors aimed to achieve 95% of patients using oxygen to have a valid prescription with target saturation range; 100% of nursing and medical staff to be trained in the safe use of oxygen to local trust/health board oxygen policy; and 90% of patients using oxygen to have oxygen signed for at the most recent drug round.

Secondary Objectives

Secondary objectives were to determine the percentage of patients receiving oxygen on four inpatient wards; the percentage of patients receiving oxygen with a valid drug chart prescription; the percentage of patients receiving oxygen with a valid drug chart oxygen prescription who have a target saturation range specified; the percentage of patients for whom oxygen saturations were documented with sufficient frequency according to their NEWS2; the percentage of patients receiving oxygen with a specified target saturation range, who had oxygen saturations actually measured within this range; and the percentage of patients who had oxygen signed for at each drug round.

METHODS

The audit was applied to adult inpatients on four wards at an acute tertiary care hospital. The wards included the acute medical unit, two respiratory wards, and the coronary care unit. Data were collected by two doctors on a single occasion on all wards in November 2017, November 2018, and November 2019, standardised using two data collection sheets validated by the BTS.^{1,2} The inclusion criteria for this audit was adult inpatients aged 18-75 years admitted to one of the four wards as described above.

There were two parts to the data that were collected. In Part 1 (Figure 1), data were collected from individual patients on each ward.



Patients using oxygen with a valid drug chart prescription

Figure 1: Percentage of patients using oxygen with a valid drug chart prescription.

This included patient identifier and ward details; oxygen status (whether oxygen was in use); whether a valid oxygen prescription was present; the number of drug rounds that took place in the past 24 hours; the number of times the drug chart was signed for oxygen in the previous 24 hours; the number of times oxygen should have been documented on the NEWS2 chart (based on the patient's score and observation level); the number of times oxygen saturations were actually recorded on the observation chart; and whether the oxygen saturation level of the patient was within their specified target range, and if not how far off.

In Part 2 (Figure 2), data collected from each ward were summarised. This included information on type of ward; date of audit; number of patients on each ward during the audit; number of patients using oxygen with drug chart prescriptions and specified target ranges; the total number of drug rounds in the past 24 hours and signatures on the drug chart for oxygen in the past 24 hours; the total number of observation rounds that should have taken place in the past 24 hours and how many oxygen saturations were actually monitored and recorded on the observation charts; the total number of patients with oxygen saturation levels within their specified target range, and if outside the range how great the deviation from the specified range; and the total number of patients with no oxygen prescription or target range.

Data from the above questionnaires were then collated, summarised, and analysed.

CHANGES IMPLEMENTED

Trust-wide changes were implemented in July 2018. In Intervention 1, changes included the introduction of NEWS2 where patients with lower target saturations had a dedicated section of the observation chart, which required verification from a registrar or above, and redesigning of the drug chart to include a tickbox specifying target oxygen saturations within the dedicated oxygen prescription section.

Changes were also implemented in August-October 2019. In Intervention 2, changes included mandatory teaching for all junior doctors on safe oxygen prescribing and guidelines; posters containing 'Oxygen is a Drug' and Oxygen Prescribing Guidelines were put up around the audited wards; and reminders were given at handover meetings for nursing staff to measure, document, and administer oxygen appropriately.



Patients with SpO2 'actually' within target range

Figure 2: Percentage of patients with oxygen saturations actually within their specified target saturations range.

RESULTS

Adult inpatients on four wards (acute medical unit, respiratory ward 1 and respiratory ward 2, and the coronary care unit) between the ages of 18 and 75 years were audited each year. There were 51 patients included in the 2017 audit, 61 patients in the 2018 audit, and 74 patients in the 2019 audit. Among these patients, 33% were using oxygen in 2017, 23% in 2018, and 19% in 2019.

Results (Table 1) showed that 86% of patients in the 2019 audit were using oxygen with a valid prescription, relative to 74% in 2018 and 77% in 2017 (Figure 1). One hundred percent of patients with a valid drug chart prescription had a target saturations range specified in the 2018 and 2019 audits, compared with 77% in 2017, which demonstrates a sustainable change and meets the expected standard set by the BTS. Furthermore, 99% of patients in 2019 had oxygen saturations documented with sufficient frequency for their NEWS2, which represents a 58% improvement from the 2018 audit (this outcome was not measured in the 2017 audit). There was a 37% increase (63% to 100%) in patients having actual oxygen saturations within their specified target saturation range in the 2019 reaudit, compared with the 2018 audit. This exceeded the National Improvement Objectives set by the BTS in 2015 (Figure 2). Finally, it appeared that fewer drug charts were being signed for by nursing staff to confirm administration of oxygen in the 2019 audit, relative to 2017 and 2018.

reaudit in November The study's 2018. following the changes implemented in July 2018 (Intervention 1), found that 26% of patients were still using oxygen without a valid prescription, 37% had saturations outside their target range, and only 12% of drug charts had oxygen signed. However, the new drug charts had ensured that all patients with valid oxygen prescriptions now had target saturations specified, representing a significant improvement from 2017. Whether oxygen saturations were being documented with sufficient frequency as per a patient's NEWS2 was additionally measured.

Table 1: Comparison of yearly audited parameters with the 2015 British Thoracic Society (BTS) audit results.

Number of patients (%)	2015 BTS audit	2017 local audit	2018 local audit	2019 local audit
Patients using oxygen with a valid drug chart prescription	58	77	74	86
Patients using oxygen with a target saturations range specified	58	77	100	100
Patients with oxygen saturations documented with sufficient frequency for their NEWS2 Score	N/A	N/A	41	99
Patients with oxygen saturations within their target saturations range	69	69	63	100
Drug charts with a valid oxygen prescription with oxygen signed for at each drug round	29	23	12	8

BTS: British Thoracic Society; N/A: not applicable; NEWS2: National Early Warning Score 2.

Based on these findings, it was recognised that not all healthcare professionals were treating oxygen as a drug, which requires appropriate prescription, monitoring, and documentation. Further changes were required to alter individual approaches to oxygen. Therefore, changes were implemented as per Intervention 2 above. The 2018 audit and its results were used to educate newly registered junior doctors of the BTS oxygen prescribing guidelines,^{1,2} and the potential dangers if not adhered to, as well as provide face-to-face and poster-based reminders to all healthcare professionals. Although there were improvements in most areas audited, the proportion of valid oxygen prescriptions completed by doctors still did not meet the 95% expected standards, and drug charts were not being appropriately signed for oxygen at each drug round.

DISCUSSION

Due to the nature of the drug and its frequent use, unfortunately, oxygen prescribing has proven to be a downfall throughout the cycles for various reasons. Poor compliance with the oxygen prescribing guidelines was found in 2017 in all areas audited. The reaudit in 2018 explored the implementation of trust-wide changes and the impact on oxygen prescribing. In the 2019 audit, 86% of patients were using oxygen with a valid prescription, compared to 74% in 2018 and 77% in 2017. Although there were improvements, the overall picture was disappointing. For an intervention to be effective past its short-term capacity, great focus should be on ongoing input from the staff on the wards in question.³

The significance of the implemented changes that were put into place following the 2018 audit enabled drastic improvements. There was a huge increase in the proportion of patients with a valid prescription for oxygen, target saturations range specified, and actual saturations within the specified range. These factors have a direct impact on patient safety and quality of care for those using oxygen in an acute setting. By improving these main areas, patient risk of hypercapnia and hypoxia have been lowered directly.⁴⁻⁶

Similar quality improvement initiatives, such as those carried out at a district general hospital in Somerset, UK, to improve compliance with oxygen prescribing guidelines, found that specifically targeting interventions to increase awareness of appropriate oxygen prescribing to the main groups of professionals who use oxygen (nursing staff and junior doctors) can have a significant impact.⁷ Another project³ highlighted that teaching sessions for junior and senior doctors and other allied healthcare prescribers can significantly improve the documentation and appropriate prescribing of oxygen. These projects further corroborate the research findings because they both implemented a similar strategy to drive improvement. However, another project⁹ found that poster reminders and multidisciplinary team education only had a

limited impact on improving oxygen prescribing. It was the use of targeted oxygen stickers above oxygen taps that ultimately enhanced oxygen prescribing practices.

There continues to be challenges around oxygen prescribing and the related consistency with signing of drug charts.^{4-6,9-11} From this study, and previous research in this area, it is has been made apparent that using focussed and targeted interventions is more likely to be able to alter attitudes around documentation and sustain this change.^{4,5,12-15} By continuously reinforcing the concept of oxygen being a drug and educating staff, the changes made can not only be sustained but also used trust-wide to positively impact patient outcome. Further targeted interventions, such as oxygen target saturation stickers or wristbands, may lead to even better compliance with the oxygen prescribing guidance and ultimately improve patient safety.

CONCLUSION

Although multiple changes were implemented over several cycles, the greatest impact was found to be from implementing staff education and awareness.^{3,7} A combination of lack of awareness, not treating oxygen as a drug, and not optimally utilising the drug chart, was exposing patients to harm. Through the amalgamation of organisational changes, and smaller local changes at each hospital, there can be an overall positive impact on patient safety, which can subsequently reduce mortality and morbidity. Simple measures such as staff education, verbal and written reminders, combined with the introduction of systemic changes such as the introduction of new drug charts, and NEWS2 scales have the greatest effect on individual behaviours. Although these trust-wide changes made it easier for oxygen saturation ranges to be documented, it is through local initiatives that each team member can reflect on alterations they could make to their practice on a personal level.

References

- O'Driscoll R. British Thoracic Society emergency oxygen audit report. National Respiratory Audit Programme. Annual Report. 2015. Available at: https://www.britthoracic.org.uk/document-library/ quality-improvement/audit-reports/ emergency-oxygen-2015. Last accessed: 29 March 2021.
- O'Driscoll BR et al. BTS guideline for oxygen use in adults in healthcare and emergency settings. Thorax. 2017;72:i1-90.
- Shaladi A et al. 53 Audited quality improvement project on oxygen prescribing on an acute surgical ward. BMJ Leader. 2019;3(Suppl 1):A20.
- 4. Cousins JL et al. 2016. Acute oxygen therapy: a review of prescribing and delivery practices. Int J Chron Obstruct Pulmon Dis. 2016;11(1):1067-75.

- Stoller JK et al. Oxygen therapy for patients with COPD. Chest. 2010;138(1):179-87.
- Brill SE, Wedzicha JA. Oxygen therapy in acute exacerbations of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis. 2014;9:1241-52.
- Helliar S. Improving oxygen prescribing rates by tailoring interventions for specific healthcare professional groups. BMJ Qual Improv Rep. 2016;5(1):u209520.w4033.
- Thein OS et al. Oxygen prescription: improving compliance using methods from BMJ Open Quality journal. BMJ Open Qual. 2018;7(2):e000288.
- Inglis A. Audit of oxygen prescribing. Treatment needs to be adjusted. BMJ. 2001;322(7289):799.

- Goldstein R. 22: The dilemma of oxygen therapy. Respiratory Medicine: COPD Update. 2007;3(1):13.
- Dodd M et al. Audit of oxygen prescribing before and after the introduction of a prescription chart. BMJ. 2000;321(7265):864-5.
- Balkissoon R. Journal club: COPD and oxygen therapy. Chron Obstr Pulm Dis. 2017;4(1):71-5.
- Bateman NT, Leach RM. ABC of oxygen. Acute oxygen therapy. BMJ. 1998;317(7161):798-801.
- COPD Foundation. Oxygen therapy. 2020. Available at: https://www. copdfoundation.org/Learn-More/Iam-a-Person-with-COPD/Oxygen-Therapy.aspx. Last accessed: 26 May 2020.
- 15. Why prescribe short-burst oxygen? Drug Ther Bull. 2007;45(9):70-2.

Prominent Cutaneous Manifestation of COVID-19: A Case Report

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Abstract

Extrapulmonary signs of coronavirus disease are becoming an important tool for patient diagnosis; this is particularly true for skin manifestations as they are visible to both the patient and physician. In this case report, the authors describe a case in which cutaneous manifestations were the most noticeable and prominent symptom in a patient with coronavirus disease.

INTRODUCTION

The new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pathogen and the subsequent coronavirus disease (COVID-19) mainly affect the lung.¹ Respiratory symptoms are key for diagnosis, however, increasing knowledge has led to the understanding that COVID-19 induces multiorgan pathology. The first descriptions of clinical symptoms reported that 0.2% of patients admitted to hospitals in China presented with a skin rash.¹ Following this, in Italy 20.4% of 88 admitted patients treated by dermatologists presented with symptoms of the skin.²

In April 2020, the first comprehensive classification of skin manifestations in patients within the full spectrum of COVID-19 severity, from intensive care, hospital wards, and home

care, as well as those without symptoms, was published.³ The study analysed 375 patients with PCR-confirmed diagnosis or with suspected diagnosis, meeting the European Centre for Disease Prevention and Control (ECDC) clinical criteria. Although following certain patterns, skin manifestations showed extreme variability. Five skin patterns were defined: pseudo-chilblain, vesicular, urticarial, maculopapular, and livedo/ necrosis. Each pattern usually associated with a different age category, evolutionary moment of the process, and systemic severity.³ The majority of these manifestations were non-specific and their cause-effect relationship with the virus is not fully established. The limited access to confirmatory tests and concurrence of different drugs to treat the disease make it difficult to reach any conclusions. The authors present the case of a patient in whom the most striking COVID-19 manifestation was dermatological.

CASE REPORT

47-year-old female, with no relevant pathological or family medical history, presented with a dry cough, diarrhoea, and chills. Three days later, she developed a headache, myalgia, and a fever (39 °C). On Day 7, she observed anosmia and improvement of the previous symptoms. On Day 16, coinciding with worsening of headache, myalgia, chills, fever, and anosmia, she presented with a skin rash, ageusia, and nausea without vomiting. The skin eruption consisted of itchy and painful lesions, first on the abdomen and later affecting the back and limbs, including the dorsum of the hands. On examination, multiple non-scaling erythematous papules and plaques were noted.

The patient was attended to in the emergency unit. Her vital signs were a temperature of 36.4 °C, blood pressure of 130/80 mmHg, heart rate of 89 beats per minute, and oxygen saturation of 98%. Her chest X-ray was reported as normal. She was discharged home and prescribed the treatment of azithromycin 500 mg every 24 hours and dexchlorpheniramine 6 mg every 8 hours for 5 days.

In the days following, the lesions became larger and oedematous with pseudo-vesicular appearance on the dorsum of the hands (Figure 1). On Day 21, she was evaluated using teledermatology. Face-to-face consultation was recommended for examination and COVID-19 testing. On examination, her temperature was 35.5 °C, oxygen saturation was 100%, chest X-ray was normal, and nasopharyngeal PCR for SARS-CoV-2 was negative. Histopathological examination showed hyperkeratosis, parakeratosis, and acanthosis. In the dermis, a dense, sleeve-like perivascular lymphohistiocytic infiltrate with abundant eosinophils pervaded throughout the dermis. Abundant eosinophils were perivascular and interstitially located. Capillaries were conspicuously dilated and engorged with red blood cells but no fibrinoid necrosis was identified (Figure 2A). Deep eccrine glands were surrounded by moderate lymphoid and eosinophil infiltration (Figure 2A). These histopathological features have previously been observed in the skin lesions of patients with COVID-19 and have been defined as having a 'mini-chilblain' pattern (Figure 2A and 2B).⁴

The patient had follow-up home visits and was treated with paracetamol, dexchlorpheniramine, and topical mometasone once per day on the lesions. In the following days, the symptoms persisted and the lesions developed a central crusty dark area.

On Day 28, the itching began to improve, and the papules flattened and turned into hyperpigmented macules concentrically surrounded by an ecchymotic halo, sparing the skin immediately adjacent to the macules. The resolution of the lesions and the itching was gradual, totally disappearing 2 months later. Three months after the onset of symptoms, the antibody test for SARS-CoV-2 was positive. Four months later, the patient presented massive shedding. The hair loss was diffuse throughout the scalp, typical of telogen effluvium; however, they also presented round bald patches with an active border, typical of alopecia areata.

DISCUSSION

COVID-19-associated skin manifestation reports are numerous; however, the authors believe that they are under-reported. There are several possible causes of this: the difficult recognition in patients with mild or absent general symptoms; the lack of knowledge regarding the manifestations of COVID-19; the difficulty accessing diagnostic tests; the clinical similarities among other dermatological diseases; and the self-resolving nature or short-term evolution. Due to the strain COVID-19 had on the healthcare system, skin examinations were not a priority among hospitalised or symptomatic patients, particularly in the most critical cases. Additionally, admission areas with restricted access made patient examination even more difficult, often preventing photographic record and obtainment of informed consent.

In COVID-19 pathogenesis, the host's response plays an important role. The first line of defence against SARS-CoV-2 is the innate immune system, specifically interferon-I production and natural killer (NK) cell intervention.⁵ The virus dampens the initial interferon-I response to achieve uncontrolled viral replication. Subsequently, humoral and cell-mediated adaptive immunity are activated.



Figure 1: Large oedematous papules on the dorsum of the hand.





Figure 2: Histological plates stained with haematoxylin and eosin.

A) 4x: Panoramic skin biopsy showing hyperkeratosis, parakeratosis, and acanthosis. In the dermis is a dense, sleevelike perivascular lymphohistiocytic infiltrate with abundant eosinophils. Eosinophils were abundant, perivascular, and interstitially located. Capillaries were conspicuously dilated and engorged with red blood cells, but no fibrinoid necrosis was identified (red arrows). Deep eccrine glands were surrounded by moderate lymphoid and eosinophil infiltration (black arrows). **B)** 20x: Photograph at high magnification showing lymphohistiocytic infiltrate with the presence of eosinophils. Inflammatory cytokines and antibodies promote the recruitment of neutrophils, monocytes, and macrophages, stimulating further development of additional inflammatory cytokines. In critical cases, activation of the coagulation pathway occurs with consumption of coagulation factors,⁶ leading to multiorgan thrombotic manifestations, including thrombosis of pulmonary and skin vessels.7 In the majority of infected patients, the immune response leads to viral clearance. However, if the effector response results in an antibody-dependent enhancement (ADE), which favours the entry of the virus into macrophages, it will result in the increase of proinflammatory cytokines such as TNF and IL-6.⁵ This phenomenon, known as cytokine storm, is the cause of tissue damage and multiorgan dysfunction and could also manifest on the skin.⁸ Nevertheless, as well as in other organs, the different manifestations of the disease in the skin must be related to the diverse immune responses of the host.9

In addition to the pattern classification described prior, another interesting study proposed a classification with two groups: exanthematic and inflammatory lesions; and vasculitic and vasculopathic lesions. In the second group, the authors differentiated the purpuric vasculitic pattern, which has a low incidence, from the petechial component in some exanthematic lesions.¹⁰ In those patterns cited, different histological and immunohistochemical findings and distinct pathophysiological hypotheses related to different host immune responses were described. A robust innate immunity response would be related to chilblain-like lesions, whereas the hypercoagulable state and cytokine storm would induce pauci-inflammatory thrombotic microangiopathy with the development of livedo or necrotic lesions.⁷ Direct viral cellular damage of angiotensin-converting enzyme 2 (ACE2) receptors on basal cells would induce the vesicular pattern. Antigen-antibody complex deposits, non-specific activation of mast cells, viral endothelial damage (rich in ACE2), complement activation, and activation of the bradykininkallikrein system have been postulated as cause of the urticarial pattern.^{11,12} In many occasions, other intercurrent causes, such as the reactivation of viral diseases or toxicoderma, cannot be ruled out.

The interest in the reported case lies in the clinical presentation of the greatly expressed skin manifestations, which is not previously described. The skin lesions were complex, longlasting, and highly symptomatic, representing the most striking symptom of the patient's symptomatology. These lesions concurred with mild general symptoms in the second week after onset and lasted for 2 months. They produced intense itching with poor response to antihistamine treatment. They evolved from small papules without scaly component to larger, oedematous, pseudo-vesicular papules resembling erythema elevatum diutinum. Later, the papules developed a central scaling with target-like images reminiscent of erythema multiforme. In the time they were flattening into macules, they presented striking concentric ecchymotic haloes. Four weeks after the disappearance of the skin lesions, intense telogen effluvium and alopecia areata appeared: two types of hair loss with different physiopathology, which do not usually appear concomitantly. No manifestations were found in mucous membranes or nails.

Taking into account the main patterns described, a maculopapular rash can be identified. Its onset coincided with the rest of the systemic symptoms, although these had already been present for 2 weeks. The effects of the medication should not be considered since the onset of the lesions was prior to the administration of any treatment. The resolution phase, with ecchymosis surrounding the lesions, cannot be explained by scratching since it had a rounded and concentric distribution around the papules and there were no linear erosions. The relationship of this sign with endothelial damage and the tendency towards hypercoagulability in patients with COVID-19 is not supported by the histological study of the biopsy. Appearance of telogen effluvium after infectious diseases is well described and the authors unreported experience supports that it is not uncommon after COVID-19.13 Alopecia areata is also already described.¹⁴ Traditionally, it has been related to immune disorders and stress; the patient reported an intense emotional burden due to the pandemic, the isolation situation, and the discomfort caused by the skin rash. Interestingly, both types of alopecia have been diagnosed more frequently after the peaks of the pandemic compared to previous periods.^{14,15}

Based on the literature reviewed, the authors assume that this patient's specific and dysregulated immune response led to her diverse and prolonged dermatological manifestations. Reporting cases is vital to expand information and provide a more precise approach to COVID-19 skin manifestations. The skin is an organ accessible to visual examination and can be extremely helpful in regard to this worldwide relevant disease.

Note: the patient consented to the presentation of the case and the images. This case is one of 375 cases in a published cohort study,³ and this case report has approval from the publishing journal.

References

- Guan W et al. Clinical characteristics of coronavirus disease 2019 in China. N Engl J Med. 2020;382(18):1708-20.
- Recalcati S. Cutaneous manifestations in COVID-19: a first perspective. J Eur Acad Dermatology Venereol. 2020;34(5):e212-3.
- Galván Casas C et al. Classification of the cutaneous manifestations of COVID-19: a rapid prospective nationwide consensus study in Spain with 375 cases. Br J Dermatol. 2020;183(1):71-7.
- Gianotti R et al. Similarities in cutaneous histopathological patterns between COVID-19-positive and COVID-19 high-risk patients with skin dermatosis. Acta Derm Venereol. 2020;100(15):1-5.
- Wang SF et al. Antibody-dependent SARS coronavirus infection is mediated by antibodies against spike proteins. Biochem Biophys Res Commun. 2014;451(2):208-14.

- Tang N et al. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost. 2020;18(4):844-7.
- Magro C et al. Complement associated microvascular injury and thrombosis in the pathogenesis of severe COVID-19 infection: a report of five cases. Transl Res. 2020;220:1-13.
- Lee N et al. Anti-SARS-CoV IgG response in relation to disease severity of severe acute respiratory syndrome. J Clin Virol. 2006;35(2):179-84.
- Criado PR et al. Lessons from dermatology about inflammatory responses in Covid-19. Rev Med Virol. 2020;30(5):e2130.
- Marzano AV et al. Cutaneous manifestations in patients with COVID-19: a preliminary review of an emerging issue. Br J Dermatol. 2020;183(3):431-42.

- Criado PR et al. What the physicians should know about mast cells, dendritic cells, urticaria, and omalizumab during COVID-19 or asymptomatic infections due to SARS-CoV-2? Dermatol Ther. 2020;33(6):e14068.
- Marzano AV et al. Varicella-like exanthem as a specific COVID-19associated skin manifestation: multicenter case series of 22 patients. J Am Acad Dermatol. 2020;83(1):280-5.
- Chu CB, Yang CC. Dengue-associated telogen effluvium: a report of 14 patients. Dermatologica Sin. 2017;35(3):124-6.
- 14. Sgubbi P et al. Alopecia areata in a patient with SARS-Cov-2 infection. Dermatol Ther. 2020;33(6):e14295.
- Kutlu Ö, Metin A. Relative changes in the pattern of diseases presenting in derma-tology outpatient clinic in the era of the COVID-19 pandemic. Dermatol Ther. 2020;33(6):e14096.

Pretransplant Determinants of Outcome in Patients with Myeloma Undergoing Autologous Transplantation in Lower Resource Settings

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Abstract

The treatment landscape in multiple myeloma has significantly changed since the introduction of high-dose melphalan with autologous stem cell rescue in the 1980s. Many randomised controlled trials have clearly demonstrated the superiority of autologous stem cell transplantation in improving survival compared to conventional chemotherapy. However, outcomes in myeloma are highly variable with median survival as short as 2 years and as long as 10 years or more. The main adverse factor predicting shorter survival is presence of high-risk cytogenetics. However, there are many other potential factors that can contribute to the treatment outcomes. This review looks at the various pretransplant variables that are associated with post-transplant outcomes in myeloma.

INTRODUCTION

Multiple myeloma is a malignant neoplasm arising from clonal proliferation of plasma cells in the bone marrow.¹ Active myeloma is defined by the presence of CRAB criteria (hypercalcaemia, renal dysfunction, anaemia, and bone lesions) and/or by the presence of biomarkers (plasma cells: ≥60%; involvedto-uninvolved serum free light chain ratio: ≥100; >1 focal lesion on MRI).² Despite so many advances in the field of myeloma and the availability of many newer potent agents, myeloma remains incurable. However, these newer agents have undoubtedly helped improve survival in myeloma to the extent that it can now be considered a chronic condition in some patients.^{3,4}

The treatment landscape in myeloma has significantly changed ever since the introduction of high-dose melphalan with autologous stem cell rescue in the 1980s. The first randomised controlled trial (RCT) by the Intergroupe Francophone du Myélome (IFM) in 1996 clearly showed the superiority of highdose melphalan followed by autologous stem cell transplantation (ASCT) over conventional chemotherapy.⁵ Since then, high-dose melphalan with stem cell transplantation has been the backbone of myeloma treatment. However, outcomes in myeloma are highly variable with median survival as short as 2 years and as long as 10 years or more.⁶ The main risk factor associated with shorter survival is presence of high-risk cytogenetics.⁶ However, there are many other potential factors that can contribute to the treatment outcomes. Hence, this review explores the various pretransplant variables that are associated with posttransplant outcomes.

BENEFIT OF AUTOLOGOUS STEM CELL TRANSPLANTATION

The results from the first two landmark clinical trials. IFM 90 trial and UK Medical Research Council (MRC) trial, have proven the superior role of ASCT in improving progressionfree survival (PFS) and overall survival (OS) compared to conventional treatment. In the IFM 90 trial, involving 200 patients, ASCT was associated with higher complete responses (CR) (22% versus 5%), 5-year event-free survival (EFS) (28% versus 10%), and higher OS (52% versus 12%).⁵ In the MRC trial, involving 401 patients, results were similar to IFM 90 with improved CR, PFS, and OS in the ASCT arm.⁷ Three later RCT, however, failed to show any survival benefit of ASCT.8-10 Differential benefits on survival from these trials have

to be addressed with caution as there was variability in the patients' age groups, induction and conditioning regimens, and the response criteria. The role of ASCT in the era of modern drugs, such as proteasome inhibitors (PI) and immunomodulatory imide drugs, was studied in four RCT, and had shown benefit in favour of ASCT in improving PFS in all four and OS in two.¹¹⁻¹⁴ In the largest study, involving 1,503 patients from multiple centres under the European Myeloma Network (EMN) group, ASCT improved response rates (≥very good partial response [VGPR] 84% versus 77%, and median PFS [57 months versus 42 months]), however, there was no OS benefit.¹⁴ A metaanalysis of the aforementioned mentioned trials using novel drugs, also concluded that there was significant improvement in PFS and a trend for benefit in terms of OS (hazard ratio for PFS: 0.55; p<0.001; hazard ratio for OS: 0.76; p=0.20).¹⁵ The main findings from the key RCT are shown in Table 1.

Table 1: Key randomised controlled trials comparing autologous stem cell transplantation with conventional chemotherapy in myeloma.

Reference	Age	N	Regimen (ASCT versus CCT)	CR (%)	PFS (median)	OS
Attal et al.,⁵ 1996	<65	200	VMCP/BVAP x 4-6 + (Mel 140 + Gy 8 TBI) versus VMCP/ BVAP x 18 cycles	22 versus 5; p<0.001	EFS at 5 years: 28 versus 10%; p=0.01	At 5 years: 52 versus 12%; p=0.03
Child et al., ⁷ 2003	<65	401	AVMpC x ≥3 + Mel 200 versus ABCM x 4-12	44 versus 8; p<0.001	31 versus 19 months; p<0.001	Median: 54 versus 42 months; p=0.04
Fermand et al., ⁸ 2005	55-65	190	VAMP x 3-4 + (Mel 200 OR BU + Mel 140) versus VMCP	36 versus 20	EFS at 25 months versus 19; p=0.07	Median: 47 versus 47 months; p=0.91
Blade et al., ⁹ 2005	<65	216	VBMCP/VBAD x 4 + (Mel 200 or Mel 140 + Gy 12 TBI) versus VBMCP/VBAD x 12	30 versus 11; p=0.002	42 months versus 33; p=NS	Median: 61 versus 66 months

Table 1 continued.

Reference	Age	N	Regimen (ASCT versus CCT)	CR (%)	PFS (median)	OS
Barlogie et al., ¹⁰ 2006	≤70	516	VAD x 4+ (Mel 140 + Gy 12 TBI) versus VAD x 4+ 1-year VBMCP ± IFN maintenance	11 versus 11	At 7 years: 17 versus 14%; p=0.16	At 7 years: 38 versus 39%; p=0.78
Palumbo et al., ¹¹ 2014	≤65	402	RD x 4 + Mel 200 versus RD x 4 + MPR x 6, ± R maintenance	16 versus 20	43 versus 22 months; p<0.001	At 4 years: 81 versus 65%; p=0.02
Gay, ¹² 2015	≤65	389	RD x 4 + Mel 200 (2) versus RD x 4 + RCD x 6, RP versus R maintenance	33-37 versus 23-27	43 versus 29 months; p<0.0001	At 4 years: 86 versus 73%; p=0.004
Attal, ¹³ 2017	≤65	700	RVD x 3 + Mel 200 + RVD x 2 versus RVD x 3 + RVD x 5, R maintenance	59 versus 48; p=0.03	50 versus 36 months; p<0.001	At 4 years: 81 versus 82%; p=NS
Cavo, ¹⁴ 2020	18-65	1,503	VCD x 3-4 + Mel 200 x 1 or 2 ± RVD x 2 + R maintenance versus VCD x 3-4 + VMP x 4 ± RVD x 2 + R maintenance	≥VGPR 84 versus 77; p=0.002	57 versus 42 months; p=0.0001	At 5 years: 75 versus 72%; p=NS

ABCM: adriamycin/carmustine/cyclophosphamide/melphalan; ASCT: autologous stem cell transplantation; AVMpC: adriamycin/vincristine/methylprednisolone/cyclophosphamide; BU: busulfan; CCT: conventional chemotherapy; CR: complete response; EFS: event free survival; IFN: interferon; Mel 140: melphalan 140 mg/m²; Mel 200: melphalan 200 mg/m²; MPR: melphalan/prednisolone/lenalidomide; NS: not significant; OS: overall survival; PFS: progression free survival; R: lenalidomide; RCD: lenalidomide/cyclophosphamide/dexamethasone; RD: lenalidomide/ dexamethasone; RP: lenalidomide/prednisolone; RVD: lenalidomide/bortezomib/dexamethasone; TBI: total body irradiation; VAD: vincristine/adriamycin/dexamethasone; VAMP: vincristine/adriamycin/methylprednisolone; VBMCP: vincristine/carmustine/melphalan/cyclophosphamide/prednisolone; VBMCP/VBAD: vincristine, carmustine, melphalan, cyclophosphamide, prednisolone/vincristine, carmustine, adriamycin, dexamethasone; VCD: bortezomib/cyclophosphamide/dexamethasone; VGPR: very good partial response; VMP: bortezomib/melphalan/ prednisolone; VMPC: vincristine/melphalan/cyclophosphamide/prednisolone; VMCP/BVAP: vincristine, melphalan, cyclophosphamide/carmustine, adriamycin, prednisolone; VMPC: vincristine, melphalan, cyclophosphamide, prednisolone; VGPR: very good partial response; VMP: bortezomib/melphalan/ prednisolone; VMPC: vincristine, melphalan, cyclophosphamide, prednisolone; VMCP/BVAP: vincristine, melphalan, cyclophosphamide, prednisolone/carmustine, adriamycin, prednisolone.

Adriamycin® (Pfizer Inc., New York City, New York, USA).

CONVENTIONAL KARYOTYPING-BASED RISK ASSESSMENT

Conventional cytogenetics reveal abnormalities roughly one-third of patients with in myeloma.^{16,17} These are mainly numerical aberrations. The low yield is caused by the low proliferative nature of plasma cells. Monosomy or deletion (g) of chromosome 13 and hypodiploidy are considered as highrisk features and are associated with inferior survival. In a report from the Mayo Clinic, 290 patients received information on conventional karyotyping: 39% had undergone ASCT and the median survival time of patients with cvtogenetic abnormalities high-risk (CA) was 29 months versus 65 months in those at standard risk (p=0.006).¹⁸ The Groupe Français de Cytogénétique Hématologique showed that among 208 patients, 75 patients had a hyperdiploidy karyotype and 63 were labelled as having hypodiploidy defined as pseudodiploid, hypodiploid, or neartetraploid chromosomes. In a multivariate analysis, the hypodiploid group had inferior survival compared to hyperdiploid group and del 13q lost its prognostic value in presence of hypodiploidy.¹⁹ Chromosome 13 abnormality retains a negative prognostic value even in patients undergoing high-dose chemotherapy and autotransplant.^{20,21} However, it is to be noted that detection of del 13g by interphase fluorescent in situ hybridisation (FISH) does not have anv negative impact on hyperdiploid myeloma.²²

FISH-BASED RISK ASSESSMENT

As a result of the poor yield by conventional karyotyping, more sensitive measures such as FISH are routinely performed for myeloma prognostication. FISH, currently considered as the standard assay, is performed on purified CD138-expressing plasma cells, or by dual-staining of cytoplasmic immunoglobulin-aided FISH.⁶ The International Myeloma Working Group (IMWG) classifies myeloma as high-risk if at least one of t(4;14), t(14;16), t(14;20), or *del 17p* is detected by FISH.²³ In addition, a gain of 1q also confers poor risk.²⁴ Bortezomib-containing induction regimen combined with ASCT can overcome the poor prognostic

impact of t(4;14) and to some extent *del 17p*. In the Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) trial, in which 25% of the patients had t(4;14), bortezomib/thalidomide/ dexamethasone (VTD) treatment could negate the poor prognostic role of t(4;14) (3-year PFS of 69% in patients with t(4;14) versus 74% without; p=0.66), whereas the thalidomide/ dexamethasone arm could not (3-year PFS of 37% in patients with t(4;14) versus 63% without; p=0.01).²⁵ Data from 354 patients with myeloma from the HOVON-65/GMMG-HD4 trial showed that bortezomib could significantly reduce adverse the impact of del 17p, which was present in 11% of patients. Patients were randomly assigned vincristine/adriamycin[®] to cycles of ([doxorubicin] Pfizer Inc., New York City, New York, USA)/dexamethasone (VAD) or bortezomib/adriamycin/dexamethasone induction followed by ASCT. In patients with del 17p, the bortezomib arm led to significant improvement in both PFS (median: 26 months versus 12 months) and 3-year OS (69% versus 17%).²⁶ Combinations of bortezomib with lenalidomide/dexamethasone^{27,28} or carfilzomib lenalidomide/dexamethasone²⁹ with are also effective in reducing the adverse impact of t(4:14) and/or del 17p on PFS in myeloma. Major CA and their impacts are summarised in Table 2.

THE REVISED INTERNATIONAL STAGING SYSTEM

The International Staging System (ISS) helps to stratify myeloma based on two parameters, albumin and β_2 -microglobulin, and categorises patients into three groups: ISS Stage I, II, and III, with median survival of 62, 44, and 29 months, respectively.³⁰ The Revised-ISS (R-ISS) was formulated by combining ISS parameters with two additional parameters, namely a lactate dehydrogenase (LDH) assay and high-risk CA. It was derived from clinical and laboratory data, pooled from 11 international trials involving 4,445 newly diagnosed patients with myeloma. R-ISS stage I was defined as ISS stage I (β_2 -microglobulin < 3.5 mg/L and albumin > 3.5 g/dL), no high-risk CA (del 17p, and/or t[4;14], and/or *t[14;16]*), and normal LDH.

Table 2: Important cytogenetic alterations in multiple myeloma.

Cytogenetic changes	Risk category	Prevalence in NDMM (%)
t(4;14) (p16;q32)/IGH-MMSET/FGFR3	Adverse	10-15
t(14;20) (q32;q12)/IGH-MAFB	Adverse	1
t(14;16) (q32;q23)/IGH-MAF	Adverse	2-5
t(6;14) (p21;q32)/IGH-CCND3	Standard	2
t(11;14) (q13;q32)/IGH-CCND1	Standard	15-20
17p deletion	Adverse	10
<i>1q21</i> gain	Adverse	35-40
<i>1p</i> deletion	Adverse	30
Hyperdiploidy	Good	50
<i>13q</i> deletion (by CK)	Adverse	45-50

CK: conventional karyotyping; NDMM: newly diagnosed multiple myeloma.

R-ISS stage III was defined as ISS stage III (β_2 -microglobulin >5.5 mg/L) and high-risk CA or high LDH. R-ISS could retain its value in delineating patients who had also undergone ASCT into the three risk groups. In total, 60% of patients had undergone ASCT and the median OS in R-ISS I, II, and III groups were 'not reached', 88 months, and 42 months, respectively.³¹

TYPE OF INDUCTION CHEMOTHERAPY

Induction chemotherapy in transplanteligible patients is given with the purpose of improving symptoms, performance status, regaining normal renal functions, correcting hypercalcaemia, and achieving an optimum response. Before the availability of novel agents, VAD was the main induction regimen for myeloma. Response rates were less, in the range 50-55%, and with very few patients achieving CR.32 Toxicity related to anthracyclines, need for continuous infusion, and the risks associated with central venous catheters were the major concerns with this regimen. Later on, efforts were made to replace doxorubicin with a liposomal form, which led to similar response rates and survival, with less toxicity.33

After the introduction of novel agents, many studies have proven superiority to the same degree. Combination of thalidomide

dexamethasone improved with response rates, both VGPR and CR, compared to VAD, before transplant.^{34,35} A meta-analysis of over 6,000 transplant-eligible patients with myeloma also reported the superiority of novel agents in improving PFS compared to the VAD regimen.³⁶ Three-drug induction regimens are more effective than two-drug regimens in improving response rates and PFS. In two RCT and one meta-analysis, VTD improved response rates and PFS compared to thalidomide/dexamethasone.^{25,36,37} In a Phase III trial comparing VTD with bortezomib/dexamethasone (VD), the former led to better response rates; however, there were no differences in PFS or OS, which could be explained by the higher numbers of patients receiving consolidation and maintenance therapy in the VD arm.³⁸ In the Southwest Oncology Group (SWOG) S0777 trial, combination of lenalidomide with VD (RVD) was compared against lenalidomide/ dexamethasone, and the RVD arm showed improved response rates, PFS, and OS.³⁹ In one of the largest retrospective studies, different induction comparing regimens, RVD led to superior response rates and OS bortezomib/cyclophosphamide/ relative to dexamethasone and VD.40

Since the introduction of next-generation PI, namely carfilzomib, which has been reported to induce very high rates of minimal/measurable residual disease (MRD) negativity,⁴¹ there has been a growing enthusiasm for substituting bortezomib with carfilzomib in the induction regimens. However, two recently published Phase III trials failed to demonstrate any advantage of carfilzomib over bortezomib with respect to response, PFS, or OS. In the ENDURANCE trial, 1,087 patients newly diagnosed with myeloma, without high-risk features, were randomised to nine cycles of induction with either carfilzomib/lenalidomide/ dexamethasone or bortezomib/lenalidomide/ dexamethasone, followed by another randomisation to lenalidomide maintenance for 2 years versus until progression or toxicity. Median PFS (35 months versus 34 months) and 3-year OS (86% versus 84%) were similar between the two groups.42 In another Phase III trial (CLARION), in elderly patients with myeloma, similar results were obtained when patients were randomly assigned bortezomib/melphalan/ to prednisolone versus carfilzomib/melphalan/ prednisolone.43 Recently, combinations of daratumumab to triple-drug induction regimens have been trialled; daratumumab was added to bortezomib/cyclophosphamide/ dexamethasone, bortezomib/lenalidomide/ dexamethasone, bortezomib/thalidomide/ dexamethasone, and carfilzomib/lenalidomide/ dexamethasone in various trials and resulted in encouraging response rates with acceptable toxicity profiles.44

RESPONSE TO CHEMOTHERAPY

Many published studies have reported that stronger responses are associated with better survival in myeloma.45 This is also applicable to responses at the end of induction therapy in patients undergoing ASCT. In a single-centre study from the UK involving 383 transplant-eligible patients with newly diagnosed multiple myeloma, achievement of partial response or CR at the end of induction therapy led to improvement in OS (median: 7.47 years in responders versus 4.89 years in nonresponders).⁴⁶ In another singlecentre study involving 211 patients, CR at transplant led to better EFS (median EFS not reached in patients with CR versus 11 months in patients with less than CR).47 At least three

more studies have shown that achieving CR or PR pretransplant was associated with improvement in both EFS/PFS and OS.48-50 These findings were further supported by a large meta-analysis of 21 ASCT studies involving nearly 5,000 patients, which showed that pretransplant maximum response (CR, near-CR, and VGPR) significantly improved both EFS/PFS and OS.⁵¹ A reduction in posttreatment fluorodeoxyglucose avidity scores positron emission tomography by was associated with improved survival outcomes. A Deauville score value of <4 of the bone marrow and focal lesions after therapy at premaintenance phase was associated with significant improvement in PFS and OS.⁵²

There is a recent growing interest in outcomes based on MRD negativity. In the MRC Myeloma IX trial, end of induction MRD negativity with multiparameter flow cytometry significantly improved PFS post-transplant, but there was no difference in OS.53 The GEM/PETHEMA study group reported that pretransplant MRD negativity by multiparameter flow cytometry resulted in a significant improvement in PFS (5-year PFS: 80% in MRD negative group versus 25% in MRD positive group; p=0.001) and a trend toward improved OS (5-year OS: 100% versus 59%; p=0.06).54 A PCRbased MRD assessment study, from marrow samples, demonstrated that low levels of MRD pretransplant is associated with an improvement in both EFS and OS. Median EFS (35 months versus 20 months; p=0.001) and OS (70 months versus 45 months; p=0.04) were significantly better in the low MRD group versus the high MRD group.⁵⁵ A meta-analysis of 21 studies, which measured response based on MRD criteria, reported that achieving MRD negativity could improve PFS and OS.⁵⁶

TIMING OF TRANSPLANT

With the availability of many highly active novel agents for myeloma treatment, the role of transplant upfront was questioned. However, in an era of both conventional and newer agents, it has been demonstrated that early transplant improves response rates and PFS. An RCT, in the era of conventional agents, compared upfront transplant (early transplant group) against conventional chemotherapy (late transplant group). A rescue transplant was considered in the late group with progression/poor response to chemotherapy. The early group showed a better response and PFS, but with no difference to OS.⁵⁷ Later, two retrospective studies, in the era of novel agents, failed to show any benefit of early transplant with respect to either PFS or OS.58,59 Gay et al.,⁶⁰ in a pooled analysis of two RCT (RV-MM-209 and EMN-441), reported that early ASCT was associated with significant improvement in PFS1 (median: 42 months versus 24 months; p<0.001), PFS2 (4 years: 71% versus 54%; p<0.001), and OS (4 years: 84% versus 70%; p<0.001), and that the benefit was seen across all prognostic subgroups.⁶⁰ In the only RCT using both lenalidomide and bortezomib in induction (IFM 2009), early transplant resulted in improved response rates (59% versus 48%), including MRD negativity, and better PFS (median 50 months versus 36 months), yet no difference in OS (at 4 years: 81% versus 82%).¹³ Despite the lack of definite OS benefit in the majority of studies, early transplantation should still be the first choice in multiple myeloma. Moreover, given the idea that achieving the maximum possible response, even to the extent of MRD negativity, can guarantee long-term survival benefit, early transplant should be offered in all possible situations.⁵⁶ Transplant at progression, even though a reasonable choice, may be hindered because of concerns over advancing age, newly acquired comorbidities, and poorer response to salvage treatment.

stem cell mobilisation is In general, recommended after 4-6 cycles of induction For mobilisation, chemotherapy. two types of regimens are commonly used: granulocyte colony-stimulating factor alone or a combination of granulocyte colonystimulating factor with cyclophosphamide. In case of inadequate mobilisation, plerixafor can be added. The specific mechanism of action of the latter, CXCR4 antagonism, helps to overcome the poor stem cell yield, associated with prolonged lenalidomide use in induction chemotherapy. Given the ongoing global coronavirus disease (COVID-19) pandemic, various national and international societies have recommended postponing autologous

transplantation, especially in patients of standard risk.^{61,62}

CONDITIONING REGIMENS

Many different conditioning regimens have been tried in myeloma over the last 20-25 years. Based on results from two prospective RCT, melphalan 200 mg/m² (Mel 200) is now the accepted standard regimen. In the IFM 9502 trial, involving 282 patients <65 years of age, Mel 200 was compared with Mel 140 + 8 Gy total body irradiation. Mel 200 was associated with faster haematologic recovery, lower degree of mucositis, shorter duration of hospitalisation, and better OS at 45 months (66% versus 45%), even though there was no difference in EFS.63 In an Italian multicentre study, Mel 200 was compared against Mel 100; the study concluded that Mel 200 resulted in improved PFS (median: 31 months versus 26 months; p=0.01) and a trend toward improved OS (at 5 years: 62 months versus 48 months; p=0.13).64 Efforts to further intensify the conditioning regimen by additional chemotherapy did not show any benefit. In an RCT by the West German Myeloma Study Group, addition of idarubicin and cyclophosphamide to Mel 200 resulted in increased toxicity, high mortality, and no significant difference in response rates or survival.65 Similarly, addition of busulfan to melphalan,66,67 or a regimen containing thiotepa, busulfan, and cyclophosphamide (compared against Mel 200) did not result in superior outcomes.68 Addition of bortezomib or carmustine with high-dose melphalan has been investigated in Phase II studies and show promising response rates; however, larger RCT are required for confirmation.^{69,70}

LIMITATIONS

Since this review has been written mainly from a practical point of view applicable to developing countries, where access to molecules like daratumumab is difficult and MRD measurement is not yet a routine practice, such things were not discussed in much detail. There is now strong evidence to suggest that achieving MRD negativity improves long-term survival and can be used as a surrogate endpoint for both PFS and OS in myeloma.^{71,72} Similarly, inclusion of anti-CD38 antibodies in the initial treatment regimen has improved PFS and OS in at least two RCT.⁷³ The author supports these changes in response assessment and treatment becoming part of routine management for patients with myeloma, including in developing countries.

CONCLUSION

High-dose melphalan followed by ASCT is the cornerstone of treatment in medically fit patients with newly diagnosed myeloma and holds value even in the era of highly active, novel agents. There are important pretransplant variables that have a role in predicting posttransplant outcomes. CA by conventional cytogenetics (*del 13q*, hypodiploidy) or

interphase FISH (*del 17p, t[4;14], t[14;16]*, t[14;20]) are associated with inferior outcomes. Triple-drug induction regimens, containing a PI and immunomodulatory imide drugs, lead to the best outcomes post-transplant with respect to response rates and survival, with this particular combination negating the adverse impact of high-risk cytogenetics to a great extent. It is ideal to have the best response at the end of induction chemotherapy, which has been found to prolong survival after transplant. An early transplant rather than at the time of relapse/ progression would be the ideal strategy, with Mel 200 as the standard conditioning regimen. Well-conducted clinical trials, especially RCT, should be considered for assessing the utility of MRD measurements in making treatment decisions, as MRD appears to be a powerful prognostic marker in myeloma.

References

- Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364:1046-60.
- Rajkumar S et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538-48.
- Kumar S et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood. 2008;111(5):2516-20.
- Costa L et al. Recent trends in multiple myeloma incidence and survival by age, race, and ethnicity in the United States. Blood Adv. 2017;1(4):282-7.
- Attal M et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Français du Myélome. N Engl J Med. 1996;335(2):91-7.
- Sonneveld P et al. Treatment of multiple myeloma with high-risk cytogenetics: a consensus of the International Myeloma Working Group. Blood. 2017;127(24):2955-62.
- Child JA et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. N Engl J. Med. 2003;348:1875-83.
- 8. Fermand JP et al. High-dose therapy and autologous blood stem-cell transplantation compared with conventional treatment in myeloma patients aged 55 to 65 years: long-term results of a randomized control trial from the group

myelome-autogreffe. J Clin Oncol. 2005;23(36):9227-33.

- Bladé J et al. High-dose therapy intensification compared with continued standard chemotherapy in multiple myeloma patients responding to the initial chemotherapy: long-term results from a prospective randomized trial from the Spanish cooperative group PETHEMA. Blood. 2005;106(12):3755-9.
- Barlogie B et al. Standard chemotherapy compared with highdose chemoradiotherapy for multiple myeloma: final results of phase III US intergroup trial S9321. J Clin Oncol. 2006;24(6):929-36.
- Palumbo A et al. Autologous transplantation and maintenance therapy in multiple myeloma. N Engl J Med. 2014;371:895-905.
- Gay F et al. Chemotherapy plus lenalidomide versus autologous transplantation, followed by lenalidomide plus prednisone versus lenalidomide maintenance, in patients with multiple myeloma: a randomised, multicentre, phase 3 trial. Lancet Oncol. 2015;16(16):1617-29.
- Attal M et al. Lenalidomide, Bortezomib, and Dexamethasone with Transplantation for Myeloma. N Engl J Med. 2017;376:1311-20.
- Cavo M et al. Autologous haematopoietic stem-cell transplantation versus bortezomibmelphalan-prednisone, with or without bortezomib-lenalidomidedexamethasone consolidation therapy, and lenalidomide

maintenance for newly diagnosed multiple myeloma (EMN02/HO95): a multicentre, randomised, open-label, phase 3 study. Erratum in: Lancet Haematol. 2020;7(6):e456-68.

- Dhakal B et al. Autologous transplantation for newly diagnosed multiple myeloma in the era of novel agent induction a systematic review and meta-analysis. JAMA Oncol. 2018;4(3):343-50.
- Calasanz MJ et al. Cytogenetic analysis of 280 patients with multiple myeloma and related disorders: primary breakpoints and clinical correlations. Genes Chromosomes Cancer. 1997;18:84-93.
- Sawyer J et al. Cytogenetic findings in 200 patients with multiple myeloma. Cancer Genet Cytogenet. 1995;82(1):41-9.
- Kapoor P et al. Evidence for cytogenetic and fluorescence in situ hybridization risk stratification of newly diagnosed multiple myeloma in the era of novel therapies. Mayo Clin Proc. 2010;85(6):532-7.
- Smadja NV et al. Hypodiploidy is a major prognostic factor in multiple myeloma. Blood. 2001;98(7):2229-38.
- Shaughnessy J et al. Continuous absence of metaphase-defined cytogenetic abnormalities, especially of chromosome 13 and hypodiploidy, ensures long-term survival in multiple myeloma treated with Total Therapy I: interpretation in the context of global gene expression. Blood. 2003;101(10):3849-56.
- 21. Shaughnessy J et al. Prognostic

impact of cytogenetic and interphase fluorescence in situ hybridizationdefined chromosome 13 deletion in multiple myeloma: Early results of total therapy II. Br J Haematol. 2003;120(1):44-52.

- 22. Chng, WJ et al. Prognostic factors for hyperdiploid-myeloma: Effects of chromosome 13 deletions and IgH translocations. Leukemia. 2006;20(5):807-13.
- 23. Rajkumar SV. Multiple myeloma: 2020 update on diagnosis, riskstratification and management. Erratum in: Am J Hematol. 2020;95(5):548-67.
- 24. Hanamura I et al. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: Incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantation. Blood. 2006;108(5):1724-32.
- 25. Cavo M et al. Bortezomib with thalidomide plus dexamethasone compared with thalidomide plus dexamethasone as induction therapy before, and consolidation therapy after, double autologous stem-cell transplantation in newly diagnosed multiple myeloma: A randomised phase 3 study. Erratum in: Lancet. 2010;376(9758):2075-85.
- Neben K et al. Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. Blood. 2012;119(4):940-8.
- Roussel M et al. Front-line transplantation program with lenalidomide, bortezomib, and dexamethasone combination as induction and consolidation followed by lenalidomide maintenance in patients with multiple myeloma: A phase II study by the Intergroupe Francophone du Myélome. J Clin Oncol. 2014;32(25):2712-7.
- Kumar S et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. Blood. 2012;119(19):4375-82.
- 29. Jakubowiak AJ et al. A phase 1/2 study of carfilzomib in combination with lenalidomide and low-dose dexamethasone as a frontline treatment for multiple myeloma. Blood. 2012;120(9):1801-9.
- Greipp PR et al. International staging system for multiple myeloma. J Clin Oncol. 2005;23(15):3412-20.
- Palumbo A et al. Revised international staging system for multiple myeloma: a report from international myeloma working group. J Clin Oncol. 2015;33(26):2863-9.

- Alexanian R et al. VAD-based regimens as primary treatment for multiple myeloma. Am J Hematol. 1990;33(2):86-9.
- 33. Rifkin RM et al. Pegylated liposomal doxorubicin, vincristine, and dexamethasone provide significant reduction in toxicity compared with doxorubicin, vincristine, and dexamethasone in patients with newly diagnosed multiple myeloma: a phase III multicenter randomized trial. Cancer. 2006;106(4):848-58.
- Macro M et al. Dexamethasone+Thalidomide (Dex/Thal) Compared to VAD as a pre-transplant treatment in newly diagnosed Multiple Myeloma (MM): A randomized trial. Blood. 2006;108(11):57.
- Cavo M et al. Superiority of thalidomide and dexamethasone over vincristine-doxorubicindexamethasone (VAD) as primary therapy in preparation for autologous transplantation for multiple myeloma. Blood. 2005;106(1):35-9.
- Sekine L et al. Frontline treatment for transplant-eligible multiple myeloma: a 6474 patients network meta-analysis. Hematol Oncol. 2019;37(1):62-74.
- Rosiñol L et al. Superiority of bortezomib, thalidomide, and dexamethasone (VTD) as induction pretransplantation therapy in multiple myeloma: a randomized phase 3 PETHEMA/GEM study. Blood. 2012;120(8):1589-96.
- Moreau P et al. Bortezomib plus dexamethasone versus reduceddose bortezomib, thalidomide plus dexamethasone as induction treatment before autologous stem cell transplantation in newly diagnosed multiple myeloma. Blood. 2011;118(22):5752-8.
- 39. Durie BGM et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. Lancet. 2017;389(10068):519-27.
- Chakraborty R et al. The impact of induction regimen on transplant outcome in newly diagnosed multiple myeloma in the era of novel agents. Bone Marrow Transplant. 2017;52(1):34-40.
- Korde N et al. Treatment with carfilzomib-lenalidomidedexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. JAMA Oncol. 2015;1(6):746-54.
- 42. Kumar SK et al. Carfilzomib or bortezomib in combination with lenalidomide and dexamethasone for patients with newly diagnosed

multiple myeloma without intention for immediate autologous stem-cell transplantation (ENDURANCE): a multicentre, open-label, phase 3, randomised, controlled trial. Lancet Oncol. 2020;21(10):1317-30.

- Facon T et al. Carfilzomib or bortezomib with melphalanprednisone for transplant-ineligible patients with newly diagnosed multiple myeloma. Blood. 2019;133(18):1953-63.
- 44. Abdallah N, Kumar SK. Daratumumab in untreated newly diagnosed multiple myeloma. Ther Adv Hematol. 2019;10:204062071989487.
- 45. Chanan-Khan AA, Giralt S. Importance of achieving a complete response in multiple myeloma, and the impact of novel agents. J Clin Oncol. 2010;28(15):2612-24.
- 46. Alvares CL et al. Long-term outcomes of previously untreated myeloma patients: responses to induction chemotherapy and high-dose melphalan incorporated within a risk stratification model can help to direct the use of novel treatments. Br J Haematol. 2005;129(5):607-14.
- 47. O'Shea D et al. Predictive factors for survival in myeloma patients who undergo autologous stem cell transplantation: a single-centre experience in 211 patients. Bone Marrow Transplant. 2006;37(8):731-7.
- Alegre A et al. Autologous peripheral blood stem cell transplantation for multiple myeloma: a report of 259 cases from the Spanish Registry. Bone Marrow Transplant. 1998;21:133-40.
- 49. Galli M et al. Feasibility and outcome of tandem stem cell autotransplants in multiple myeloma. Haematologica. 2005;90(12):1643-9.
- 50. Björkstrand B et al. Prognostic factors in autologous stem cell transplantation for multiple myeloma: an EBMT registry study. Leuk Lymphoma. 1994;15(3-4):265-72.
- 51. Van De Velde HJK et al. Complete response correlates with longterm survival and progression-free survival in high-dose therapy in multiple myeloma. Haematologica. 2007;92(10):1399-406.
- 52. Zamagni E et al. Standardization of 18 F-FDG-PET/CT according to Deauville Criteria for metabolic complete response definition in newly diagnosed Multiple Myeloma. J Clin Oncol. 2018;39(2).
- 53. Rawstron AC et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX study. J Clin Oncol. 2013;31(20):2540-7.
- 54. Paiva B et al. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo
autologous stem cell transplantation. Blood. 2008;112(10):4017-23.

- Korthals M et al. The level of minimal residual disease in the bone marrow of patients with multiple myeloma before high-dose therapy and autologous blood stem cell transplantation is an independent predictiveparameter. Biol Blood Marrow Transplant. 2012;18(3):423-31. e3.
- Munshi NC et al. Association of minimal residual disease with superior survival outcomes in patients with multiple myeloma: a meta-analysis. JAMA Oncol. 2017;3(1):28-35.
- 57. Fermand JP et al. High-dose therapy and autologous peripheral blood stem cell transplantation in multiple myeloma: up-front or rescue treatment? Results of a multicenter sequential randomized clinical trial. Blood. 1998;92(9):3131-6.
- 58. Kumar SK et al. Early versus delayed autologous transplantation after immunomodulatory agents-based induction therapy in patients with newly diagnosed multiple myeloma. Cancer. 2012;118(6):1585-92.
- 59. Dunavin NC et al. Early versus delayed autologous stem cell transplant in patients receiving novel therapies for multiple myeloma. Leuk Lymphoma. 2013;54(8):1658-64.
- 60. Gay F et al. Autologous transplant vs oral chemotherapy and lenalidomide in newly diagnosed young myeloma patients: a pooled analysis. Leukemia. 2017;31(8):1727-34.
- 61. American Society of Hematology. COVID-19 and Multiple Myeloma: frequently asked questions. 2021.

Available at: https://www.hematology. org/covid-19/covid-19-and-multiplemyeloma. Last accessed: 1st February 2021.

- 62. The European Society for Blood and Marrow Transplantation. COVID-19 and BMT: EBMT RECOMMENDATIONS. 2021. Available at: https://www.ebmt.org/covid-19and-bmt. Last accessed: April 2021.
- Moreau P et al. Comparison of 200 mg/m2 melphalan and 8 Gy total body irradiation plus 140 mg/ m2 melphalan as conditioning regimens for peripheral blood stem cell transplantation in patients with newly diagnosed multiple myeloma: final analysis of the Intergroupe Francophone du Myélome 9502 randomized trial. Blood. 2002;99(3):731-5.
- 64. Palumbo A et al. Melphalan 200 mg/m2 versus melphalan 100 mg/ m2 in newly diagnosed myeloma patients: a prospective, multicenter phase 3 study. Erratum in: Blood. 2010;115(10):1873-9.
- 65. Fenk R et al. High-dose idarubicin, cyclophosphamide and melphalan as conditioning for autologous stem cell transplantation increases treatmentrelated mortality in patients with multiple myeloma: Results of a randomised study. Br J Haematol. 2005;130(4):588-94.
- 66. Lahuerta JJ et al. Conditioning regimens in autologous stem cell transplantation for multiple myeloma: A comparative study of efficacy and toxicity from the Spanish Registry for transplantation in multiple myeloma. Br J Haematol. 2000;109(1):138-47.
- 67. Blanes M et al. intravenous busulfan

and melphalan as a conditioning regimen for autologous stem cell transplantation in patients with newly diagnosed multiple myeloma: a matched comparison to a melphalanonly approach. Biol Blood Marrow Transplant. 2013;19(1):69-74.

- 68. Anagnostopoulos A et al. Comparison of high-dose melphalan with a more intensive regimen of thiotepa, busulfan, and cyclophosphamide for patients with multiple myeloma. Cancer. 2004;100(12):2607-12.
- 69. Lonial S et al. A phase I/II trial combining high-dose melphalan and autologous transplant with bortezomib for multiple myeloma: a dose- and schedule-finding study. Clin Cancer Res. 2010;16(20):5079-86.
- Chen AI et al. Tandem chemomobilization followed by high-dose melphalan and carmustine with single autologous hematopoietic cell transplantation for multiple myeloma. Bone Marrow Transplant. 2012;47(4):516-21.
- Munshi NC et al. A large metaanalysis establishes the role of MRD negativity in long-term survival outcomes in patients with multiple myeloma. Blood Adv. 2020;4(23):5988-99.
- 72. Medina A et al. Comparison of next-generation sequencing (NGS) and next-generation flow (NGF) for minimal residual disease (MRD) assessment in multiple myeloma. Blood Cancer J. 2020;10(108).
- Korst CLBM et al. Should all newly diagnosed MM patients receive CD38 antibody-based treatment? Hematology Am Soc Hematol Educ Program. 2020;2020(1):259-63.

Rifampicin-Induced Thrombocytopenia: A Case Report and Short Review of the Literature

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Abstract

Thrombocytopenia may be associated with a variety of conditions and risks depending on its severity, ranging from mild epistaxis to life-threating bleeding. Many drugs or herbal remedies can cause thrombocytopenia by either inhibiting platelet production and/or enhancing their destruction from the peripheral blood mediated via an immunological mechanism implicating drug-dependent antibodies. The latter entity is called drug-induced immune thrombocytopenia: a life-threatening, under-recognised condition, which is often a diagnostic challenge. Rifampicin is a widely used, well-tolerated, and effective bactericidal drug. Adverse events, except for gastrointestinal effects, headache, skin rash, and pruritus, are uncommon. The authors herein report on a patient with isolated thrombocytopenia with a recent medical history of brucellosis on rifampicin and doxycycline. Thrombocytopenia was proved to be rifampicin-induced. Also presented is a short review of the literature on this rare subject, which should be of great importance to clinicians.

INTRODUCTION

Thrombocytopenia is generally defined as a platelet count below the lower normal limit, <150x10⁹/L, although many suggest that a cutoff value of 100x10⁹/L is more appropriate to identify clinically significant thrombocytopenia.¹ It may be associated with a variety of conditions and risks ranging from mild epistaxis to lifethreating bleeding. At the time of initial presentation, thrombocytopenia may be isolated or combined with other cytopenias. The cause of thrombocytopenia may be unclear,² and the clinician is usually faced with distinguishing among various possible pathologies such as sepsis, disseminated intravascular coagulation, microangiopathic processes, or autoimmune disease.^{3,4} Many drugs and components, including nutritional supplements or herbal remedies,⁵ can also cause thrombocytopenia by either inhibiting platelet production and/or enhancing their destruction from the peripheral blood mediated via drug-induced immune thrombocytopenia (DITP).⁶

DITP is a life-threatening, under-recognised condition, and is often a diagnostic challenge.⁷ Detection of drug-dependent antibodies (DDabs) in a patient's serum or plasma with suspected DITP is often not possible. This is untrue for heparin-induced-thrombocytopenia, for which the detection of antibodies against platelet factor 4-heparin complex can confirm the suspected diagnosis. Most patients with DITP have multiple comorbidities and other potential causes of thrombocytopenia;⁷ thus, patients with DITP are often diagnosed with idiopathic immune thrombocytopenic purpura. It should be noted that heparin-induced-thrombocytopenia is a special case of drug-induced thrombocytopenia with a mechanism unrelated to other DITP.

Rifampicin is a widely used, well-tolerated, and effective bactericidal drug. It is the most commonly used drug for nontuberculous mycobacterial disease but may also be part of antituberculosis or antibrucellosis regimens, or serve as prophylaxis following exposure to *Neisseria meningitidis* or *Haemophilus influenzae*. Adverse events are uncommon, except for gastrointestinal effects, headache, skin rash, and pruritus.

Brucellosis is a zoonotic disease with a wide clinical spectrum. Haematological abnormalities have been seen in brucella-infected patients and the most encountered are anaemia and leukopenia.⁸ Isolated thrombocytopenia has been reported in up to 8% of cases by Akdeniz et al.,⁹ whereas immune-mediated thrombocytopenia has also been reported.^{9,10} The authors of this case report describe a patient with isolated thrombocytopenia with a recent medical history of brucellosis treated with rifampicin and doxycycline. Thrombocytopenia was proved to be rifampicin-induced. They also present a short review of the literature on this rare subject.

CASE PRESENTATION

A 38-year-old male was referred to the Department of Haematology in the hospital of the authors' place of work for thrombocytopenia. He had a medical history of brucellosis 3 months previously and he had been administered rifampicin 900 mg daily and doxycycline 100 mg twice per day. He had stopped the prescribed treatment before the completion of the recommended period of 6 weeks.

One week before his admission, he was evaluated in another hospital because of malaise, lowgrade fever, and fatigue, symptoms compatible with his previous history of incompletely treated brucellosis. The patient was administered the same antibiotic medication of rifampicin and doxycycline. During his hospitalisation, he presented with ecchymoses on both upper limbs and petechial rash on the oral mucosa. His complete blood count was normal except for low platelets (14x10⁹/L). The patient was started on corticosteroid therapy for a presumed diagnosis of immune thrombocytopenic purpura.

Upon admission, the patient's platelet count was 44x10⁹/L. Corticosteroid therapy was continued while the rest of his medication was stopped. A differential work-up for his thrombocytopenia was initiated. The peripheral blood smear confirmed isolated thrombocytopenia with no morphological abnormalities in the erythroid or myeloid lineage. Prothrombin time and activated partial thromboplastin time were within normal limits. The patient was negative for hepatitis C, HIV, Epstein-Barr virus, cytomegalovirus, and helicobacter pylori (breath test). Antinuclear antibodies were also negative and serum immunoglobulins were within normal range. On the same day, a bone marrow aspiration with cultures was conducted with no abnormal findings. The following day, doxycycline and rifampicin were reintroduced. A critical decrease in the number of platelets was noted from 44x10⁹/L to 7x10⁹/L. A clinical suspicion of drug-induced thrombocytopenia prompted the discontinuation of rifampicin, which resulted in a rapid increase in the number of platelets. Unfortunately, laboratory tests for DDAbs were not available in the authors' hospital.

The patient showed clinical improvements during his hospitalisation. IgM-specific antibodies for brucella and bone marrow culture were negative and IgG-specific antibodies for brucella were positive. For this reason, discontinuation of doxycycline was also decided after discharge. The patient was discharged after 5 days, having no symptoms and a platelet count of 106x10⁹/L (Figure 1). Upon follow-up 1-week postdischarge, the patient's clinical examination was unremarkable, and the platelet count was 315x10⁹/L. The patient was advised to avoid future use of rifampicin.

Platelet levels during hospitalisation



Figure 1: Platelet levels during hospitalisation in a patient with rifampicin-induced thrombocytopenia.

This figure illustrates the levels of platelets (PLT; $x10^{9}/L$) during hospitalisation. Rifampicin was stopped on Day 1 and was resumed on Day 3 (79 $x10^{9}/L$). The next day, because of a significant decrease in the platelet count (7 $x10^{9}/L$), rifampicin was stopped again, and the number of platelets increased rapidly thereafter. Follow-up 1 week postdischarge showed unremarkable clinical examination and platelet count of 315 $x10^{9}/L$.

DISCUSSION

The authors of this review present a patient rifampicin-induced thrombocytopenia. with The considered differential diagnoses in this case were vast: pseudothrombocytopenia was eliminated because there was no platelet aggregation in the peripheral blood smear; myelodysplastic syndrome aplastic or anaemia were excluded after evaluation of the peripheral blood and bone marrow. Thrombotic thrombocytopenic purpura was considered but there were no schistocytes in the peripheral blood smear, there were no symptoms of anaemia, and renal function, bilirubin, and lactate dehydrogenase were normal; disseminated intravascular coagulopathy eliminated was because the clotting screen and fibrinogen were normal. Common variable immunodeficiency was considered but Ig levels were within normal limits; infections were examined and the patient was negative for hepatitis C, HIV, Epstein-Barr virus, cytomegalovirus, and helicobacter pylori. Lastly,

autoimmune disease was disregarded because antinuclear antibodies were also negative and the patient did not report arthralgias, sun sensitivity, skin rash, or mouth ulcers.

There are certain criteria that need to be fulfilled to prove a causal relationship between thrombocytopenia and a candidate drug, as suggested by George et al. in 1998.¹¹ These criteria are as follows: 1. Therapy with the candidate drug must precede thrombocytopenia, and recovery from thrombocytopenia must be complete and sustainable with the discontinuation of the drug; 2. The candidate drug must be the only one used before the onset of thrombocytopenia, or other drugs must be continued or reintroduced after discontinuation of therapy with the candidate drug with a sustained normal platelet count; 3. Other causes should be excluded; 4. Re-exposure to the candidate drug results in recurrent thrombocytopenia.¹¹

In this present case, rifampicin was a candidate drug causing thrombocytopenia

and the discontinuation of the drug led to the resolution of thrombocytopenia. Other causes of thrombocytopenia were excluded, such as normal bone marrow, negative screening for viruses, and autoimmune diseases, and reintroduction of the candidate drug caused a recurrent decrease of platelets. In addition, the proposal of thrombocytopenia caused by other drugs was excluded, because doxycycline was continued when the treatment of rifampicin was stopped and there were no other drugs administered. This patient, therefore, fulfilled all four criteria required, leading to a definitive level of evidence (Level I) that thrombocytopenia was drug-induced. The Adverse Drug Reaction Probability Scale, developed by Naranjo et al.,¹² was used with a score of 9 (Definite).

Limited epidemiologic data exist for DITP. An estimated incidence of approximately 10 cases per 1,000,000 in the population per year has been reported,¹³ although this is likely to be an underestimate. DITP can be caused by two different mechanisms: suppression of platelet production (myelosuppressive drugs and drugs that affect the metabolism of folic acid) and platelet destruction. Destruction of platelets can be induced by at least three different mechanisms, which include the hapten-dependent antibodies, the platelet-reactive autoantibodies, the DDabs, or both drug-dependent and drug-specific antibodies.^{14,15} Hapten-dependent antibodies bind covalently to the protein of the thrombocyte membrane and this complex induces immunemediated cell destruction. The second mechanism encompasses antibody binding against the platelet membrane, while the third mechanism of DDabs is not well understood.^{14,15} Bougie et al.¹⁵ proposed a model in which the administration of the drug increased the affinity between DDabs and the epitope on a platelet glycoprotein.

DITP induced by DDabs is classically caused by antibodies inducing platelet destruction by the reticuloendothelial system only in the presence of the drug.¹⁶ Quinine was the first drug recognised as being involved in this group of DITP; however 'quinine-type' antibodies typically recognise a restricted set of epitopes on glycoprotein Ib/IX¹⁷ and glycoprotein IIb/ Illa complexes of the platelet membrane.¹⁸ Rifampicin-induced thrombocytopenia has been linked to the presence of antiplatelet antibodies, which have higher avidity for platelet membrane in the presence of rifampicin.¹⁹ The epitope of rifampicin-dependent antibodies is on glycoprotein IX, a subunit of the glycoprotein IX complex.²⁰ An experimental study showed that rifampicin-dependent antibodies also targeted glycoprotein llb/llla.²¹ Currently available laboratory tests can identify the causative agent in patients for whom there is suspicion and clinical evidence of DITP, including rifampicin-induced thrombocytopenia.^{22,23} However, the aetiology of DITP can be established when a prompt rise in platelets occurs with the discontinuation of the drug, while its reintroduction leads to a new drop in platelet count, as noted in this patient.

Rifampicin-induced thrombocytopenia was first described by Blajchman et al.²⁴ in 1970. Since then, almost 40 cases have been documented in the literature and a recent review by Cooper and Ghanima³ included rifampicin in the group of common drugs that cause immune-mediated thrombocytopenia.³ Case reports and series of rifampicin-induced thrombocytopenia are presented in Table 1.

Rifampicin-induced thrombocytopenia usually occurs when the drug is either intermittently administered or reintroduced after a voluntary discontinuation, as is the case in this present study.^{25,26} Bansal et al.²⁷ described a case of rifampicin-induced thrombocytopenia after reexposure to rifampicin 10 years later. It has been suggested that drug sensitivity and induction of thrombocytopenia remains indefinitely.57 In four cases in the literature, thrombocytopenia previous exposure to occurred with no rifampicin,²⁸⁻³¹ whereas in three more, reported by Lee et al.,³² thrombocytopenia occurred following a 4-month period of rifampicin prophylaxis in a tuberculosis outbreak.32

Corticosteroids have been used in cases where thrombocytopenia was the result of an immune-mediated mechanism; their benefit is not proven.⁵⁸ Transfusion of platelets is indicated when a patient presents with severe thrombocytopenia or wet purpura owing to a great risk of intracranial haemorrhage.^{14,33} Plasmapheresis and intravenous Ig have been used in drug-induced thrombocytopenia.^{59,60} All treatment options are controversial. The most crucial step is the immediate discontinuation of the drug. Table 1: Cases of rifampicin-induced thrombocytopenia in literature.

Reference	Sample size (n)	Study design
Mehta et al., ¹⁹ (1996)	3	Case report
Agrawal et al., ²² (2012)	1	Case report
Blajchman et al., ²⁴ (1970)	1	Case report
Verma et al., ²⁵ (2010)	1	Case report
Kindelán et al.,²6 (1994)	1	Case report
Bansal et al., ²⁷ (2013)	1	Case report
Bashir et al., ²⁸ (2016)	1	Case report
Lee et al., ²⁹ (1989)	2	Case report
Pau et al., ³⁰ (1987)	1	Case report
Esposito et al., ³¹ (1971)	1	Case report
Lee et al., ³² (2012)	87 (who received rifampicin)	Cohort study (n=3.4%)
Kang et al., ³³ (2010)	1	Case report
Nair et al., ³⁴ (2009)	1	Case report
Mauricio et al., ³⁵ (2019)	1	Case report
Arnold et al., ³⁶ (2015)	2,500*	Observation study
Yakar et al., ³⁷ (2013)	1	Case report
Dixit et al., ³⁸ (2012)	1	Case report
Mori et al., ³⁹ (2011)	1	Case report
Davis et al., ⁴⁰ (2009)	6+	Case report
Kant et al.,41 (2008)	1	Case report
Muñoz et al., ⁴² (2008)	1	Case report
Scognamiglio et al.,43 (2008)	2 (1 received rifampicin)	Case report
Garg et al., ⁴⁴ (2007)	1	Case report
Krivoy et al.,45 (2001)	1	Case report
Pereira et al., ⁴⁶ (2000)	1	Case report
Tricerri et al.,47 (1997)	1	Case report
Martínez et al.,48 (1994)	1	Case report
Juang et al., ⁴⁹ (1992)	1	Case report
Bhasin et al., ⁵⁰ (1991)	1	Case report
Burnette et al., ⁵¹ (1989)	1	Case report
Kakaiya et al., ⁵² (1989)	1	Case report
Hadfield ⁵³ (1980)	1	Case report
Poole et al., ⁵⁴ (1971)	49‡	Clinical trial
Ferguson ⁵⁵ (1971)	1	Case report
Leggat ⁵⁶ (1971)	1	Case report

*Thrombocytopenia (121/1,000 infant days)

⁺Thrombocytopenia (n=1)

‡Thrombocytopenia (n=3)

The reintroduction of the drug is not indicated even if small doses are to be used;²⁵ however, Nair et al.³⁴ supported the continuation of rifampicin along with treatment for immune thrombocytopenia. Should be aware of its life-threatening risks. Thrombocytopenia can be complicated with mild bleeding events such as epistaxis or bruising but also with severe events such as gastrointestinal bleeding and acute subdural

CONCLUSION

Although thrombocytopenia is a rare side effect of rifampicin, healthcare professionals

should be aware of its life-threatening risks. Thrombocytopenia can be complicated with mild bleeding events such as epistaxis or bruising but also with severe events such as gastrointestinal bleeding and acute subdural haemorrhage.³³ Notably, previous exposure to the drug is not necessary for the presentation of thrombocytopenia. Patients should be advised to avoid future use of the drug in order to prevent the potential detrimental effects of thrombocytopenia.

References

- Smock KJ, Perkins SL. Thrombocytopenia: an update. Int J Lab Hematol. 2014;36(3):269-78.
- 2. Arnold DM, Lim W. A rational approach to the diagnosis and management of thrombocytopenia in the hospitalized patient. Semin Hematol. 2011;48(4):251-8.
- Cooper N, Ghanima W. Immune thrombocytopenia. N Engl J Med. 2019;381(10):945-55.
- Rousan TA et al. Recurrent acute thrombocytopenia in the hospitalized patient: sepsis, DIC, HIT, or antibioticinduced thrombocytopenia. Am J Hematol. 2010;85(1):71-4.
- Ohmori T et al. Acute thrombocytopenia induced by jui, a traditional herbal medi-cine. J Thromb Haemost. 2004;2(8):1479-80.
- Aster RH et al. Drug-induced immune thrombocytopenia: pathogenesis, diagnosis, and management. J Thromb Haemost. 2009;7(6):911-8.
- Arnold DM et al. Approach to the diagnosis and management of druginduced immune thrombocytopenia. Transfus Med Rev. 2013;27(3):137-45.
- al-Eissa Y, al-Nasser M. Haematological manifestations of childhood brucellosis. Infection. 1993;21(1):23-6.
- Akdeniz H et al. Hematological manifestations in brucellosis cases in Turkey. Acta Med Okayama. 1998;52(1):63-5.
- Gurkan E et al. Immune thrombocytopenic purpura associated with Brucella and Toxoplasma infections. Am J Hematol. 2003;74(1):52-4.
- George JN et al. Drug-induced thrombocytopenia: a systematic review of published case reports. Ann Intern Med. 1998;129(11):886-90.
- Naranjo CA et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30(2):239-45.

- Chong BH et al. Drug-induced immune thrombocytopenia. Hematol Oncol Clin North Am. 2013;27(3):521-40.
- van den Bemt PMLA et al. Druginduced immune thrombocytopenia. Drug Saf. 2004;27(15):1243-52.
- Bougie DW et al. Patients with quinine-induced immune thrombocytopenia have both "drugdependent" and "drug-specific" antibodies. Blood. 2006;108(3):922-7.
- Vayne C et al. Pathophysiology and diagnosis of drug-induced immune thrombocytopenia. J Clin Med. 2020;9(7):2212.
- Burgess JK et al. Quinine-dependent antibodies bind a restricted set of epitopes on the glycoprotein Ib-IX complex: characterization of the epitopes. Blood. 1998;92(7):2366-73.
- Peterson JA et al. Fine specificity of drug-dependent antibodies reactive with a restricted domain of platelet GPIIIA. Blood. 2008;111(3):1234-9.
- Mehta YS et al. Rifampicin-induced immune thrombocytopenia. Tuber Lung Dis. 1996;77(6):558-62.
- Burgess JK et al. Rifampicindependent antibodies bind a similar or identical epitope to glycoprotein IX-specific quinine-dependent antibodies. Blood. 2000;95(6):1988-92.
- Sun P et al. Rifampicin-dependent antibodies target glycoprotein IIb/ IIIa and cause clearance of human platelets in NOD/SCID mice. Br J Haematol. 2016;172(1):137-40.
- Agrawal A et al. Do not miss rifampicin-induced thrombocytopenic purpura. BMJ Case Rep. 2012;2012:bcr1220115282.
- 23. Arnold DM et al. A systematic evaluation of laboratory testing for drug-induced immune thrombocytopenia. J Thromb Haemost. 2013;11(1):169-76.
- Blajchman MA et al. Rifampicininduced immune thrombocytopenia. Br Med J. 1970;3(5713):24-6.

- 25. Verma AK et al. Rifampicin-induced thrombocytopenia. Indian J Pharmacol. 2010;42(4):240-2.
- Kindelán JM et al. Rifampin-induced severe thrombocytopenia in a patient with pulmonary tuberculosis. Ann Pharmacother. 1994;28(11):1304-5.
- Bansal R et al. A case of thrombocytopenia caused by rifampicin and pyrazinamide. Indian J Pharmacol. 2013;45(4):405-7.
- Bashir H et al. Rifampcin-induced thrombocytopaenia purpura. Indian J Chest Dis Allied Sci. 2016;58(3):189-90.
- 29. Lee CH, Lee CJ. Thrombocytopenia--a rare but potentially serious side effect of ini-tial daily and interrupted use of rifampicin. Chest. 1989;96(1):202-3.
- 30. Pau AK, Fisher MA. Severe thrombocytopenia associated with once-daily rifampin therapy. Drug Intell Clin Pharm. 1987;21(11):882-4.
- 31. Esposito R, Vitali D. Rifampicin and thrombocytopenia. Lancet. 1971;2(7722):491-2.
- Lee SH et al. Adverse events and development of tuberculosis after 4 months of rifampicin prophylaxis in a tuberculosis outbreak. Epidemiol Infect. 2012;140(6):1028-35.
- 33. Kang SY et al. Acute subdural hemorrhage associated with rifampicin-induced thrombocytopenia. Neurol Sci. 2010;31(2):199-200.
- 34. Nair S, Carroll WD. Carry on doctor. Arch Dis Child. 2009;94(9):734-5.
- Maurício J et al. Severe rifampicininduced thrombocytopenia in a patient with miliary tuberculosis. Pulmonology. 2019;26(4):247-9.
- Arnold CJ et al. Rifampin use and safety in hospitalized infants. Am J Perinatol. 2015;32(6):565-70.
- Yakar F et al. Isoniazidand rifampicin-induced thrombocytopenia. Multidiscip Respir Med. 2013;8(1):13.

- Dixit R et al. Thrombocytopenia due to rifampicin. Lung India. 2012;29(1):90-2.
- Mori M et al. A case of acute and severe thrombocytopenia due to readministration of rifampicin. J Infect Chemother. 2011;17(2):288-90.
- 40. Davis LC et al. Meningococcal disease in a backpackers hostel in Scotland: a risk assessment for prophylaxis. Euro Surveill. 2009;14(31):19291.
- Kant S et al. Rifampicin, ethambutol and pyrazinamide-induced thrombocytopenia. Int J Clin Pharmacol Ther. 2008;46(8):440-2.
- 42. Muñoz ME et al. Rifampin-related acute renal failure, thrombocytopenia, and leuko-cytoclastic vasculitis. Ann Pharmacother. 2008;42(5):727-8.
- Scognamiglio F et al. Flow cytometry in the diagnosis of druginduced thrombocytopenia: two illustrative cases. Am J Hematol. 2008;83(4):326-9.
- 44. Garg R et al. Rifampicin induced thrombocytopenia. Indian J Tuberc. 2007;54(2):94-6.
- 45. Krivoy N et al. Rifampin-induced thrombocytopenia: diagnosis by a novel in vitro lymphocyte toxicity assay. Isr Med Assoc J. 2001;3(7):536-8.

- Pereira J et al. Glycoprotein Ib/IX complex is the target in rifampicininduced immune thrombocytopenia. Br J Haematol. 2000;110(4):907-10.
- Tricerri A et al. Adverse drug reaction to rifampin: a case with long lasting antiplatelet antibodies. Panminerva Med. 1997;39(1):64-6.
- Martínez E et al. Evidence implicating rifampin-independent antiplatelet antibodies in the pathogenesis of rifampin-induced immune thrombocytopenia. Clin Infect Dis. 1994;19(2):351-3.
- 49. Juang YC et al. Acute renal failure and severe thrombocytopenia induced by rifampicin: report of a case. J Formos Med Assoc. 1992;91(4):475-6.
- Bhasin DK et al. Can rifampicin be restarted in patients with rifampicininduced thrombocytopenia? Tubercle. 1991;72(4):306-7.
- Burnette PK et al. Rifampinassociated thrombocytopenia secondary to poor compliance. DICP. 1989;23(5):382-4.
- 52. Kakaiya RM et al. Rifampin-induced immune thrombocytopenia. A case report. Vox Sang. 1989;57(3):185-7.
- Hadfield JW. Rifampicin-induced thrombocytopenia. Postgrad Med J. 1980;56(651):59-60.

- Poole G et al. Potentially serious side-effects of high-dose twiceweekly rifampicin. Postgrad Med J. 1971;47(553):727-47.
- 55. Ferguson GC. Rifampicin and thrombocytopenia. Br Med J. 1971;3(5775):638.
- Leggat PO. Rifampicin and thrombocytopenia. Lancet. 1971;2(7715):103-4.
- 57. Aster RH et al. Drug-induced immune thrombocytopenia. N Engl J Med. 2007;357(6):580-7.
- Pedersen-Bjergaard U et al. Druginduced thrombocytopenia: clinical data on 309 cases and the effect of corticosteroid therapy. Eur J Clin Pharmacol. 1997;52(3):183-9.
- Ray JB et al. Intravenous immune globulin for the treatment of presumed quinidine-induced thrombocytopenia. DICP. 1990;24(7-8):693-5.
- Pourrat O. Treatment of drug-related diseases by plasma exchanges. Ann Med Interne (Paris). 1994;145(5):357-60.

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