Vol. 5.4 December 2020

EMJ

European Edition

+ INTERVIEW:

PROF ILONA KICKBUSCH

Championing Global Health

+ EDITOR'S PICK

Primer on the Pathogenesis of Severe COVID-19: Part One

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Contents

"We hope that this issue of EMJ contributes to this rich tapestry of global knowledge, helping to combat COVID-19 and enhance medical advancement for better patient care worldwide."

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VIEW IN FULL ←

Aims and Scope

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EMJ is published four times a year. For subscription details please visit: www.emjreviews.com

ISSN 2397-6764

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EMJ 5.4

Welcome

Dear Readers,

Welcome to our latest issue of *EMJ*! I am proud to share with you our cross-specialty flagship eJournal, bringing valuable insights across a range of disciplines and reflecting the collaborative nature of not only clinical medicine, but of the spirit of medical progress and global research that is a hallmark of 2020. Understanding of the current coronavirus disease (COVID-19) pandemic has drawn on insights shared by every medical discipline, and further analysed across laboratories, public health services, and pharmaceutical development. We hope that this issue of *EMJ* contributes to this rich tapestry of global knowledge, helping to combat COVID-19 and enhance medical advancement for better patient care worldwide.

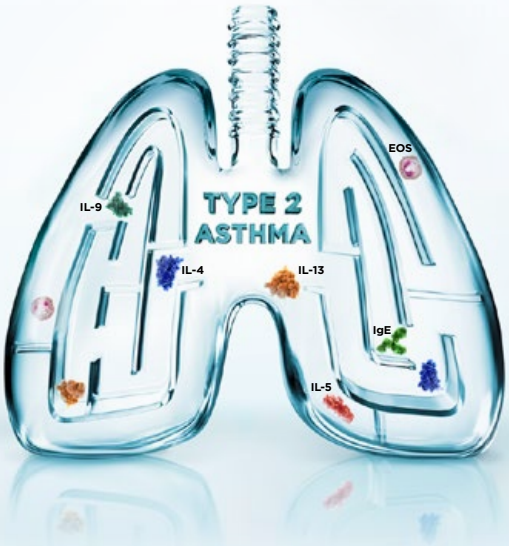
Our Editor's Pick is a timely and detailed appraisal of the current understanding of the pathophysiology in severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). In a two-part comprehensive manuscript, Walsh discusses immune cell biology and virology features that shape the manner in which COVID-19 manifests, in an excellent primer for every clinician.

Other articles shared in this issue span topics from the cellular level, all the way to health service screening recommendations. Volkmann et al. share a detailed discussion on the evolution of gastrointestinal tract symptoms in systemic sclerosis in their article 'Longitudinal Characterisation of the Gastrointestinal Tract Microbiome in Systemic Sclerosis.' Mason et al. consider the value of 'prehabilitation' to improve treatment success in patients with rheumatoid arthritis in 'Optimising Response to Advanced Therapies in Rheumatoid Arthritis – Using Prehabilitation to Improve Success?'. Meanwhile, Lee et al. contribute a detailed review of the therapy landscape for the management of advanced urothelial carcinoma in 'Changing Paradigms in the Treatment of Advanced Urothelial Carcinoma: A 2020 Update'.

Whatever your daily scope of practice, I am sure you will find the cross-discipline insights shared in the following pages to be interesting and impactful. I am proud of the role that EMJ plays in distributing research within the global clinical and research community in our open-access, high-quality eJournals, and I wish to thank our expert authors, dedicated editorial board, and editorial team for their contributions. I hope you enjoy the fascinating and important research inside this flagship issue of *EMJ*.

Spencer Gore

Chief Executive Officer, EMG-Health



PRESENT IN ~50%[a] TO 70%[b] OF YOUR ADULT ASTHMA PATIENTS,

TYPE 2 INFLAMMATION IS HIGHLY HETEROGENEOUS AND A PREDICTOR OF RISK FOR FUTURE EXACERBATIONS[1-4]

IDENTIFY

Type 2 inflammation in asthma

HETEROGENEITY

Encompasses several phenotypes[2]:

- Allergen-driven
- Mixed eosinophilic and allergen-driven
- Eosinophilic

SIMPLE IDENTIFICATION

Identifiable by one or more of the following criteria[5]:

- ✓ Elevated EOS
- ✓ Allergen-driven
- ✓ Elevated FeNO
- ✓ OCS-dependency

EOS, eosinophils; **FeNO**, fractional exhaled nitric oxide; **OCS**, oral corticosteroid.

TARGET

Cytokines IL-4, IL-5 and IL-13 are key drivers of type 2 inflammation in asthma[6-8]

	IL-4	IL-13	IL-5
Th2 cell differentiation	●		
B-cell class switching and IgE production	●	●	
Eosinophil recruitment and trafficking to tissue	●	●	●
Eosinophil differentiation in bone marrow			●
Mucus production and goblet cell hyperplasia		●	
Smooth muscle hypertrophy and tissue remodeling	●	●	

IL-4 and IL-13 have distinct and overlapping roles with a broad impact on asthma symptoms[7,8]

TREAT

TO REDUCE

Exacerbations

Oral corticosteroids

TO IMPROVE

Lung function

Quality of life

Target and treat type 2 inflammation holistically to achieve optimal asthma control[1,5]

[a]N=205.
[b]N=37.

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Foreword

Dear Colleagues,

It is my pleasure to welcome you to the 5.4 issue of *EMJ*. The overarching theme for this edition is the understanding of disease pathophysiologic mechanisms to enable targeted therapeutic strategies and individualised precision medicine. A variety of peer reviewed articles, spanning a number of disease areas, are presented.

My Editor's Pick is the paper 'Primer on The Pathogenesis of Severe COVID-19' by Walsh, a timely must read for all. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) inactivates antiviral activities by initially muting IFNβ production, leading to impaired T-cell and antibody responses. Further, its robust activation of protein kinase R (PKR)/PKR-like endoplasmic reticulum kinase, which is mutually exclusive to NLRP3 stimulation, facilitates viral replication and IL-1β production. Subsequent amplification of IFNβ expression causes dysregulation of IL-6 production which, in the T-cell depleted milieu, results in the cytokine storm response. On the other hand, virally induced NADPH oxidase activity and an oxidant environment intensifies proinflammatory milieu and is associated with severe organ damage. This understanding of disease pathophysiology will help to direct future therapeutic strategies.

Other disease areas include optimisation of targeted therapies in rheumatoid arthritis, checkpoint immunotherapy in urologic cancer, and prostate cancer surveillance. The role of ethnicity in chronic heart failure and intraoperative cardiac arrest are also reviewed, as well as data on gastrointestinal microbiome profiles in scleroderma and clinical profiles and outcomes in paediatric CNS infections.

As highlighted in this issue, advances in disease pathophysiology are driving targeted novel therapeutic approaches and precision medicine, extending the boundaries for disease management and patient outcomes.

With this wealth of content, I am very pleased to present *EMJ 5.4* and thank all the authors and peer-reviewers for committing time to this eJournal despite the pressures of the COVID-19 crisis.

With kind regards,

Prof Ian C. Chikanza

Department of Rheumatology, St Bartholomew's Arthritis Centre, St Bartholomew's and The Royal London Hospital, London, UK

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Surrounding Advanced Heart Failure: The Role of the Latest Left Ventricular Assist Devices

This symposium took place on 9th October 2020, as part of the virtual 34th European Association for Cardio-Thoracic Surgery (EACTS) Annual Meeting

Chairpeople: Ivan Netuka,[1] Daniel Zimpfer[2]

Speakers: Ivan Netuka,[1] Daniel Zimpfer,[2] Ana González,[3] Marie-Cécile Bories,[4] Ramzi Abi Akar[4]

1. Cardiovascular Surgery Department, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
2. Medical University of Vienna, Vienna, Austria
3. Hospital Puerta de Hierro, Madrid, Spain
4. Hôpital Européen Georges-Pompidou (Hôpital Européen Georges Pompidou AP-HP), Paris, France

Disclosure: Prof Netuka has served as a surgical proctor and consultant for Abbott; a principal investigator and advisory board member for Carmat SA; and has served as an advisory board member and is a stockholder for Leviticus Cardio Ltd., Virginia Israel Advisory Board, and Evaheart, Inc. Prof Zimpfer has been an advisor and proctor for Abbott and Berlin Heart. Dr González has received consultancy fees from Abbott. The other speakers have declared no conflicts of interest.

Acknowledgements: Medical writing assistance was provided by Dr Pelle Stolt, Basel, Switzerland.

Support: The symposium and publication of this article were funded by Abbott. The views and opinions expressed are those of the speakers and not necessarily of Abbott.

Citation: EMJ. 2020;5[4]:12-18.

Meeting Summary

Advanced stage heart failure accounts for 1–10% of the overall heart failure population and the prevalence is increasing, in part because of improved treatment options, which have led to longer life expectancy in today's patients compared to those of a generation ago. Recently, major improvements in technology and in the understanding of risk profiles have led to advancements in the use of mechanical circulatory support (MCS) devices, most notably the left ventricular assist devices (LVAD). The present article summarises key data, insights, and experiences recently presented by an expert panel at the virtual symposium 'Surrounding Advanced Heart Failure', held during the European Association for Cardio-Thoracic Surgery (EACTS) Annual Meeting 2020. The symposium focussed on how today's LVAD therapies fit into the cardiological continuum, how to minimise the risk of adverse events, and how the impact of the coronavirus disease (COVID-19) pandemic might be mitigated by novel treatment approaches.

Introduction

Over the last half century, the morbidity and mortality of patients with congestive heart failure have been greatly improved by disease-modifying drugs and innovative device therapies; and yet, heart failure remains a progressive disease. Patients have progressed towards advanced-stage heart failure, a condition that currently accounts for 1–10% of the overall population of patients with heart failure. Improved treatment has contributed to an increased prevalence, as today's patients live longer than those of the previous generation.[1] Device therapies have long been an option for patients with advanced heart failure, but in the last decade there have been seismic shifts in the treatment landscape. Several clinical trials and registries have confirmed a large improvement in the risk/benefit profile of MCS devices, in particular LVAD. Recently, these advancements were counterbalanced by the advent of the COVID-19 pandemic, which has reduced access to care for high-risk groups, particularly end-stage patients awaiting elective surgery. The pandemic has generated new challenges for the multidisciplinary teams involved in the care of vulnerable patients.

Selecting Candidates for Ventricular Assist Device Therapy

In the early stages of heart failure, disease-modifying therapies and symptom management are important to slow progression and preserve quality of life. However, at the transition to advanced heart failure, oral pharmacotherapy starts to fail, the patients' quality of life deteriorates markedly, and major treatment decisions are required. The options of heart transplant or MCS, either temporary (extracorporeal membrane oxygenation [ECMO]) or long-term (LVAD), all require careful evaluation.

Technology improvements have made a profound difference to patients' survival chances in the last decades. Two-year survival on an assist device was 23% in 2001 (8% on optimal medical management),[2] whereas the latest generation of LVAD has a reported 2-year survival rate of 79%, rivalling the rate for heart transplants (82%) (Figure 1).[2-6] Postmarket studies with LVAD have confirmed the greatly improved survival: 83% at 2 years in the ELEVATE registry of HeartMate 3™ LVAD (Abbott, Abbott Park, Illinois, USA).[7,8] This is an extremely longed-for development, as transplantation has always been a limited therapeutic option for patients with end-stage chronic heart failure. Despite the continuing advances, selection of appropriate candidates for LVAD therapy remains difficult and the decision requires a multidisciplinary approach. As with any interventional therapy, it is important to identify comorbidities that should be considered carefully, as well as the 'sweet spot', when patients are neither too ill nor too well to derive meaningful benefits from treatment. The optimal LVAD candidates are those expected to have poor outcomes without intervention and favourable outcomes with the intervention, and who are not contraindicated.[9] Ideally, patients should be referred at an early disease stage to reduce the risk associated with the procedure.

The easiest decision for an LVAD is with patients identified as New York Heart Association (NYHA) Class IV who are haemodynamically stable but need low or intermediate doses of inotropes because of hypotension, worsening of symptoms, or progressive renal failure.[10] For more severe, as well healthier, patients, the decision needs to account for the risk of adverse events with the therapy. Fortunately, these are becoming less frequent with newer device generations.

Improved Adverse Event Profile of Modern Left Ventricular Assist Devices

The associated risk starts with the implant procedure. Here, modern devices are increasingly designed for less invasive operations. One method relies on bilateral mini-thoracotomy in the fourth or fifth left intercostal space and the second right intercostal space. This grants access to the LV apex, as well as to the ascending aorta.[11] The less invasive method preserves the pericardium and seemingly requires less intraoperative blood products.[11] The approach has further been shown to be associated with reduced rates of postoperative right ventricular failure,[12] one of the most common and serious complications of LVAD therapy.[13]

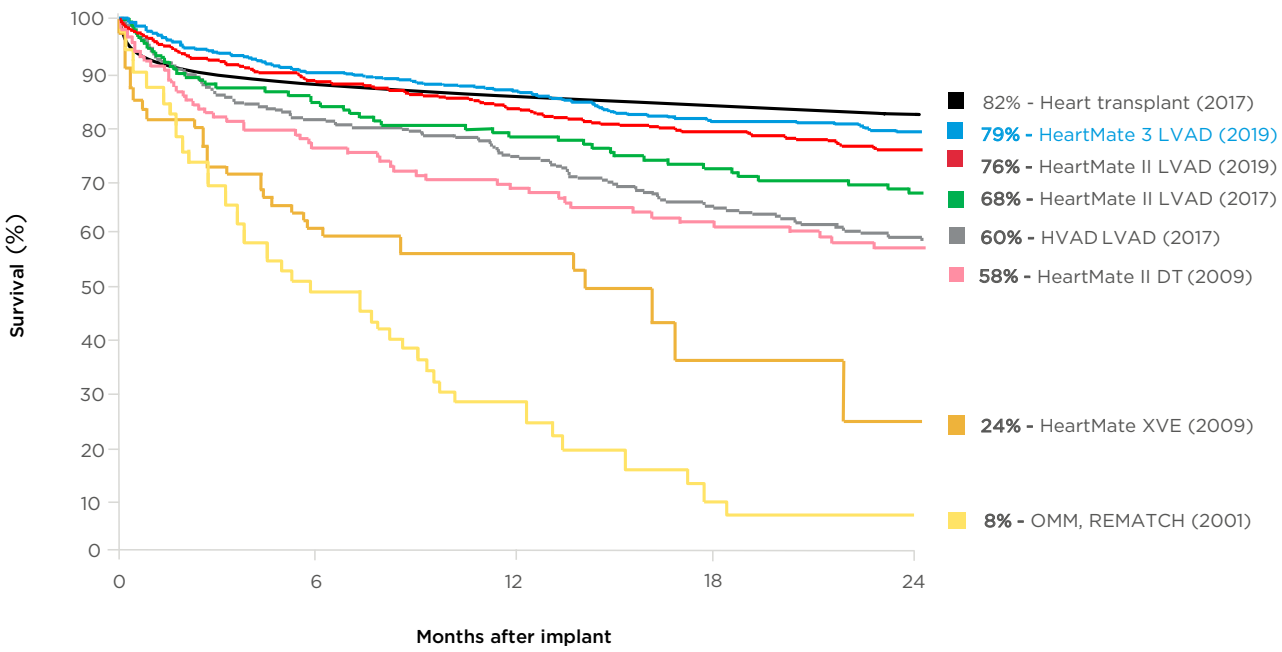

Figure 1: Survival with different generations of left ventricular assist devices compared with medical management.

A historical perspective of the survival of patients with end-stage heart failure who partook in randomised clinical trials. The yellow lines depict the survival associated with optimal medical therapy and pulsatile technology. Eight years after the completion of the REMATCH trial,[2] pulsatile technology was replaced in favour of smaller continuous flow pumps, which translated into not only a dramatic improvement in survival as seen in the purple, green, and red curves, but also a drastic improvement in quality-of-life metrics. This new HeartMate (Abbott, Abbott Park, Illinois, USA) technology is associated with a 2-year survival of 83%, which now parallels that of heart transplantation.

DT: destination therapy; HVAD: HeartWare® [Medtronic, Dublin, Ireland] left ventricular assist device; LVAD: left ventricular assist device; OMM: optimal medical management.

Adapted from Rose et al.,[2] Lund et al.,[3] Mehra et al.,[4] Rogers et al.,[5] and Slaughter et al.[6]

Recently, Rieband et al.[11] showed that patients who had experienced less invasive LVAD implantation had better outcomes in subsequent heart transplants. In addition to the reduced need for blood products, there was less formation of antibodies against the donated heart than in patients operated on in the traditional way.[11]

Beyond the implant procedure, patients may be at risk of device malfunction, infection, bleeding, or stroke, with sometimes fatal consequences.[13] This was particularly true for earlier LVAD devices and development efforts in recent years have focussed on reducing the risk of adverse events. Today, there is good evidence of lower rates of suspected pump thrombosis and stroke with the latest generation HeartMate 3. In the randomised MOMENTUM 3 trial[5] and the international, real-world, all-comers ELEVATE registry,[8] rates of suspected pump thrombosis and stroke were lower than in earlier LVAD generations (Table 1). The results were highly similar in both the randomised trial and in real-world use; rates of gastrointestinal bleeding in real-life use were less than one-half of those in the controlled trial.

A comparison of adverse event rates with different types of devices was published in the latest annual report from the Society of Thoracic Surgeons (STS) Interagency Registry for Mechanically Assisted Circulatory Support (Intermacs).[7] In the analysis, patients who had received a centrifugal flow with a full magnetic levitation device (HeartMate 3) had the highest rates of freedom from first stroke, gastrointestinal bleeding, and major infection at 12 months, and the trend continued at 18 months.

Table 1: Two-year adverse event rates in the ELEVATE registry and the MOMENTUM 3 trial.

	ELEVATE N=463		MOMENTUM 3		
			HM3	HM II	HM3
Adverse event	**n (%)**	**EPPY**	**n (%)**	**n (%)**	**EPPY**
Suspected pump thrombosis	7 (1.5)	0.009	7 (1.4)	70 (13.9)	0.01
Any stroke	45 (9.7)	0.059	51 (9.9)	98 (19.4)	0.08
Haemorrhagic stroke	24 (5.2)	0.031	25 (4.9)	43 (8.5)	0.03
Ischaemic stroke	21 (4.5)	0.028	29 (5.6)	65 (12.9)	0.04
Disabling stroke	-	-	26 (5.0)	38 (7.5)	0.04
Any bleeding	155 (33.5)	0.355	225 (43.7)	278 (55.0)	0.61
Requiring surgery	57 (12.3)	0.091	50 (9.7)	89 (17.6)	0.08
Not requiring surgery	-	-	197 (38.3)	251 (49.7)	0.53
Gastrointestinal bleeding	45 (9.7)	0.079	126 (24.5)	156 (30.9)	0.31

EPPY: events per patient-year; HM3: HeartMate 3™ left ventricular assist device (Abbott, Abbott Park, Illinois, USA); HM II: HeartMate II™ left ventricular assist device (Abbott).

Adapted from Mehra et al.4 and Zimpfer at al.[8]

Given the limited follow-up for the newest devices, comparisons beyond 1 year are limited and data from longer follow-up will be necessary to confirm the trends.

Netuka et al.[14] have been investigating the possibility of decreasing anticoagulant use to reduce the risk of bleeding without increasing that of pump thrombosis or stroke. Results are available from an initial open-label trial in 15 patients who received a vitamin K antagonist at the target international normalised ratio 2.0–3.0 for 6 weeks after implant of a HeartMate 3 and who were then transitioned to a lower international normalised ratio target range of 1.5–1.9. After 6 months, 93% remained free from pump thrombosis, disabling stroke, or major bleeding.[14] Larger studies are ongoing, including an investigation as to whether antiplatelet therapy can be withdrawn while maintaining vitamin K antagonist therapy.

Adverse events have not been eliminated and it is important to address them using a team approach; a dedicated team including cardiologists and referral centres should be involved. The establishment of Networks of Excellence is highly encouraged.

Use of Modern Left Ventricular Assist Devices

Originally considered only as a lifesaving therapy for patients ineligible for heart transplantation, LVAD are now indicated for bridge-to-transplant, bridge-to-recuperation, or as destination therapy. As noted above, patients in a severe condition may be too ill to benefit from LVAD. Patients requiring urgent transplant have significantly worse survival than elective transplants.[15] However, it has been shown that temporary ECMO may improve the severity score of patients who are very ill, making them more suitable LVAD candidates. In a study that stabilised patients in severe conditions with ECMO before implanting an LVAD, mid-term survival after implant was comparable to that of less ill patients.[16]

In alignment with these findings, guidelines have recommended that unstable patients with cardiogenic shock should receive ECMO support before considering further therapies (bridge-to-decision).[17] If the neurological function is favourable, the stabilised patient can be referred for long-term or bridge-to-therapy LVAD. However, for stabilisation before transplant in patients with advanced heart failure, ECMO may not be the best option. This has been

shown by Crespo-Leiro et al.[1] in critically ill, profoundly haemodynamically compromised patients with end-organ dysfunction in urgent need of transplantation. The best option was temporary bridging with LVAD, which was associated with significantly higher survival rates than bridging with ECMO or temporary biventricular assist devices.[18] This corresponds to the recommendations in current heart failure guidelines.[10]

Left Ventricular Assist Devices in a COVID-19 World

The COVID-19 pandemic has upended healthcare systems worldwide, either as a result of the 'lockdown' measures or by directing resources towards infected patients. During lockdown, care delivery systems were reorganised in unprecedented ways, particularly in the cardiovascular community. There was a dramatic reduction in the availability of donor hearts: there were fewer car accidents, additional safety measures aimed at excluding potentially asymptomatic severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) carriers, and fewer non-COVID-19 patients admitted to intensive care units.[19] As advanced heart failure is a condition with high morbidity and mortality, the situation raised urgent questions about how to treat these vulnerable patients during the crisis.

Given the severity of the situation, Dr Bories and Dr Akar considered the possibility of LVAD therapy, which is readily available and has a good survival rate at 1 year. Additionally, the operation is elective, meaning surgical and hospital capacity can be planned in advance. The team chose to implant LVAD in six patients with severe heart failure with left monoventricular dysfunction. The objective was to stabilise their condition and keep them safe at home, as well as to reduce the duration of hospitalisation and thus minimise their exposure to the virus. One of the patients had developed irreversible and refractory cardiogenic shock 1 month after SARS-CoV-2 infection; the others had negative virus tests (Table 2).

All six HeartMate 3 implantations were successful, with five of six patients discharged within 30 days of the procedure; survival at the time of writing was 100%. The postoperative status of the patient infected with SARS-CoV-2 was uncomplicated. Importantly, none of the other five patients contracted the virus during or after implantation. This may have been because of the strict social distancing, limited family visits, and strict precautions taken by the caregivers. Follow-up has been continued through telemedicine methods and physical visitation.

The experience showed that, in spite of the COVID-19 pandemic, cardiologists still have the tools to provide appropriate therapies to patients. LVAD may be the treatment of choice for advanced heart failure because of the lack of donor hearts.

Conclusions

The management of advanced heart failure is evolving at a rapid pace. Pulling together the new knowledge and wide experience from recent years will require a multidisciplinary effort. Many questions on LVAD therapy remain; high on the wish list is to minimise the risks associated with the implant procedure and subsequent infection. By learning how to avoid right ventricular failure, how best to manage bleeding versus thrombosis risk, and what blood pressure levels to target, current LVAD devices will be able to provide maximal benefits in the most suitable patients. If future technological advancements are of the same magnitude as those of the last 20 years, the outlook for patients with advanced heart failure will be transformed.

Table 2: Characteristics and outcomes of patients receiving left ventricular assist devices during the national lockdown in France.

Patient	1	2	3	4	5	6
Age/sex	51/Male	33/Female	39/Male	57/Male	59/Male	67/Male
Aetiology of heart failure	Ischaemic	Ischaemic	DCM	DCM	DCM	Ischaemic
Intermacs	4	4	2	3	3	1 (veno-arterial ECMO)
Preoperative COVID-19 real-time PCR	Negative	Negative	Negative	Positive	Negative	Negative
Intended goal of pump support	Bridge-to-candidacy	Bridge-to-transplant	Bridge-to-transplant	Bridge-to-transplant	Bridge-to-transplant	Bridge-to-decision
Intensive care unit length of stay before LVAD implantation	2	10	9	12	20	29
Duration receiving LVAD support outside hospital (days)	29	18	22	30	21	72
Follow-up (3 months)	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing	Ongoing

COVID-19: coronavirus disease; DCM: dilated cardiomyopathy; ECMO: extracorporeal membrane oxygenation; Intermacs: Interagency Registry for Mechanically Assisted Circulatory Support; LVAD: left ventricular assist device.

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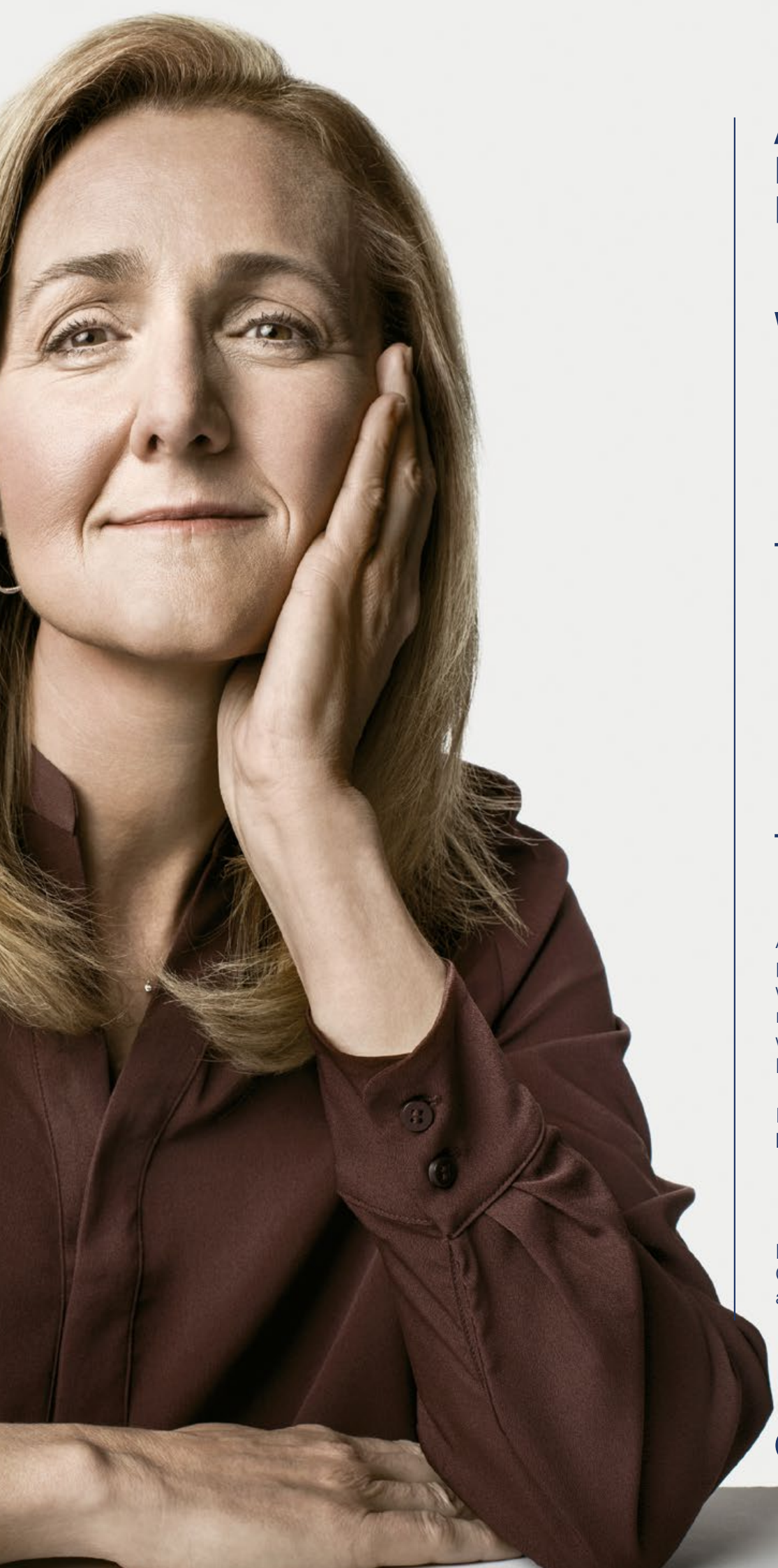

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Synbiotics in Cow's Milk Allergy Management: Inspiration, Effect on Outgrowth, and Impact on Immunity Against Infectious Diseases

This symposium took place on 16th October 2020, as part of the virtual Food Allergy and Anaphylaxis Meeting and European Consortium on Application of Flow Cytometry in Allergy (FAAM–EUROBAT) Digital 2020

Chairperson: Nikos Papadopoulos[1,2]

Speakers: Jan Knol,[3,4] Kirsten Beyer,[5] Anna Nowak-Wegrzyn[6,7]

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Disclosure: Prof Papadopoulos has received personal fees from Novartis, Nutricia, HAL Allergy Group, Menarini/Faes Farma, Sanofi, Mylan/Meda, biomay, AstraZeneca, GlaxoSmithKline, MSD, ASIT Biotech, Boehringer Ingelheim; and grants from Gerolymatos International SA and Capricare. Prof Knol is a full-time Director of the Gut Biology & Microbiology Platform of Danone Nutricia Research, the Netherlands. Prof Beyer has received research grants from Aimmune Therapeutics, ALK, Stiftung Berliner Sparkassen, Danone Nutricia, DBV Technologies, DST Diagnostic, GoodMills, HiPP, Hycor Biomedical, InfectoPharm, Thermo Fisher Scientific, VDI; has received other research support from the European Union (EU), German Research Foundation (DFG), Federal Ministry of Education and Research (BMBF), Federal Ministry of Food and Agriculture (BMEL); has been on the speakers bureau or received honoraria from Aimmune Therapeutics, Allergopharma, Bencard Allergie, Danone Nutrica, DiText, Hammer & Rall Media, InfectoPharm, Mylan/Meda, Nestle, Thermo Fisher Scientific; and has been a consultant for or a member of the advisory board for Aimmune Therapuetics, ALK, Bausch + Lomb, Bencard Allergie, Danone Nutrica, DBV Technologies, HiPP, Hycor Biomedical, InfectoPharm, Mabylon, Mylan/Meda, Nestle, Novartis. Prof Nowak-Wegrzyn has received honorarium for this presentation from Nutricia; and has received a research grant and has been a site principal investigator for the clinical study (PRESTO) of amino acid-based formula with synbiotics.

Acknowledgements: Medical writing assistance was provided by Jennifer Taylor, London, UK.

Support: The publication of this article was funded by Nutricia Specialized Nutrition.

Citation: EMJ. 2020;5[4]:20-28.

Meeting Summary

This symposium took place during the virtual Food Allergy and Anaphylaxis Meeting and European Consortium on Application of Flow Cytometry in Allergy (FAAM–EUROBAT) Digital 2020, hosted by the European Academy of Allergy and Clinical Immunology (EAACI). Focussing on the use of synbiotics to manage infants with cow's milk allergy (CMA), the speakers discussed the importance of the gut microbiota, and the impact of synbiotics on CMA outgrowth and reported infection outcomes. Prof Knol set the scene by describing how infants with CMA often have an altered gut microbiota compared to healthy breastfed infants. He explained the composition of synbiotics and presented data from three randomised controlled trials (RCT) that have demonstrated how synbiotics rebalanced the gut microbiota in infants with CMA, bringing them closer to that of healthy breastfed infants. Prof Beyer discussed cow's milk as a common food allergen, and how children often develop tolerance naturally. She then presented results from the PRESTO trial, which showed that approximately one-half of children with IgE-mediated CMA who received an amino acid-based formula (AAF), with or without synbiotics, developed cow's milk tolerance within 12 months. The results were in line with what has been reported in the literature for this population. Prof Nowak-Wegrzyn highlighted how simple infections can have a detrimental effect on young children and lead to treatment with antibiotics. Infants and children with CMA are more susceptible and prone to recurrent infections. Three RCT have suggested that an AAF with synbiotics can reduce infections and the use of antibiotics in children with IgE-mediated CMA and non-IgE-mediated CMA. The meeting concluded with a panel discussion.

Synbiotics: Inspiration from Human Milk and the Effect on the Gut Microbiota

Professor Jan Knol

Early life is a critical time for the development of the gut microbiota and maturation of the immune system. The infant gut is almost sterile at birth and needs to be colonised to form the gut microbiome. The immune system is also naïve at birth and needs to learn to distinguish between good and dangerous signals from the environment. These developmental processes go hand in hand.

The gut hosts 70–80% of the human body's immune cells,[1] and its microbiota support immune function and development.[2-5] There is also crosstalk between the gut microbiota and the immune system.[2,3] The gastrointestinal (GI) mucosal immune system plays a pivotal role in the maintenance of immune homeostasis and is crucial for suppressing responses to harmless antigens and beneficial bacteria, as well as responding to threats such as toxins or pathogenic bacteria.

The gut microbiota acts as a barrier against pathogens. Imbalances of the gut microbiota, known as dysbiosis, can trigger several immune disorders through the activity of T cells, such as allergic reactions and infections.[6,7] In contrast, a healthy, balanced gut microbiota acts as a barrier against the infiltration and colonisation of pathogens, thereby protecting infants against infections at the epithelial layer.[8-10] A number of mechanisms are at play in the healthy gut to prevent the growth of pathogens. Beneficial bacteria compete with pathogens for adhesion sites and nutrients in order to produce antimicrobial peptides and bacterial metabolites such as short chain fatty acids (SCFA). The acidic environment hinders pathogen growth and enables healthy bacteria, such as bifidobacteria and lactobacilli, to thrive. Healthy bacteria also support the integrity of the epithelial and mucosal barrier with tight junctions and production of mucus.

Multiple factors can impact the gut microbiota in early life and potentially cause dysbiosis.[11] These include the maternal microbiota and duration of gestation, with preterm infants having an aberrant microbiome development. Mode of delivery also plays a role, as infants born via caesarean section have a different exposure to microbes compared to those delivered vaginally. Other factors include early dietary feeding (breast milk versus formula), use of antibiotics and/or probiotics, and environmental factors such as family size and exposure to pets.

Infants with CMA often have an altered gut microbiota compared to healthy breastfed infants.[12] Healthy infants have dominant levels of *Bifidobacterium* species, *Faecalibacterium prausnitzii*, and *Lactobacillus* species. In infants with allergies, these numbers are lower and there is a greater prevalence of adult-like, potentially pathogenic bacteria, such as *Enterococcus faecalis*, *Clostridium difficile*, and Campylobacter.

Breastfeeding is the preferred nutrition for infants and is undisputedly the best nourishment for all infants worldwide. It also contains many components, such as live bacteria, prebiotic oligosaccharides, and lactose, which all stimulate the growth of beneficial bacteria and the healthy development of the immune system. When breastfeeding is not possible, hypoallergenic formulas are recommended to manage infants with CMA. However, traditional hypoallergenic formulas, including extensively hydrolysed formula and AAF, lack the microbiota-stimulating factors of breast milk.

This is where the synbiotic concept comes into play, providing the best of both worlds. Synbiotics are a combination of prebiotics (the substrates for growth of beneficial bacteria) plus probiotics (live bacteria that, when administrated in adequate amounts, confer a health benefit).[13-16] This blend of pre- and probiotics mimics the composition of human breast milk and the two ingredients work synergistically to target gut microbiota dysbiosis.[17]

Human breast milk contains thousands of different oligosaccharides,[18] 162 of which have been identified.[19] These oligosaccharides have multiple functions but of most importance is the ability to act as a substrate for the growth of bacteria in babies' GI tract, i.e., prebiotics. The benefits of prebiotics have been acknowledged in the World Allergy Organization (WAO)–McMaster University Guidelines for Allergic Disease Prevention (GLAD-P).[20] Multiple studies have been conducted on the specific prebiotic mixtures' short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS) and short-chain fructo-oligosaccharides and lcFOS (scFOS/lcFOS) in 9:1 ratios. These blends mimic the diversity, quantity, and functionality of human milk oligosaccharides in human breast milk.[21-24]

A synbiotic is created by taking the prebiotic and adding the probiotic *Bifidobacterium breve* M-16V® (Morinaga Milk Industry, Tokyo, Japan), a unique infant strain. *B. breve* is one of the most commonly isolated *Bifidobacterium* species from human breast milk.[2,25] It is a natural species in the infant gut and is one of the predominant *Bifidobacterium* species in breastfed infants.[26,27] The M-16V strain was selected for its ability to reduce allergic responses, as shown in preclinical[28,29] and clinical studies,[30,31] and for its proven safety.[32] For example, M-16V induces no undesired antibiotic resistances and is free from major allergens (cow's milk, eggs, wheat, nuts, peanut, soya, and fish/shellfish protein).

Six RCT on either extensively hydrolysed formula or AAF including synbiotics have now been published, demonstrating the safety and effectiveness in healthy, high-risk, and allergic infants. An extensive clinical trial programme has been conducted on an AAF with synbiotics (*B. breve* M-16V with scFOS/lcFOS) over more than 10 years, of which three specifically studied infants with CMA. A study by Harvey et al.[33] showed that the formula was safe, well tolerated, promoted normal growth in healthy infants, and was hypoallergenic according to the American Academy of Pediatrics (AAP) guidelines. A study by Burks et al.[34] demonstrated that an AAF with synbiotics was safe and well tolerated in infants with IgE-mediated CMA and non-IgE-mediated CMA. Meanwhile, a study by Candy et al.[35] in infants with non-IgE-mediated CMA showed that synbiotics were able to balance the gut microbiota, bringing it closer to the gut microbiota seen in breastfed infants after 8 weeks. The ongoing PRESTO trial is investigating oral tolerance to cow's milk and the incidence of future allergies in infants with IgE-mediated CMA.

Importantly, three RCT have shown that synbiotics rebalance the gut microbiota in infants with CMA so that it more closely resembles that of healthy breastfed infants (PRESTO, unpublished data; Figure 1).[34-36] Intervention with synbiotics increased beneficial bifidobacteria while reducing levels of bacteria more commonly seen in adults, namely members of the *Eubacterium rectale/Clostridium coccoides* genera. The gut environment also shifted to a healthier state, with increased levels of SCFA and a lower pH.[37]

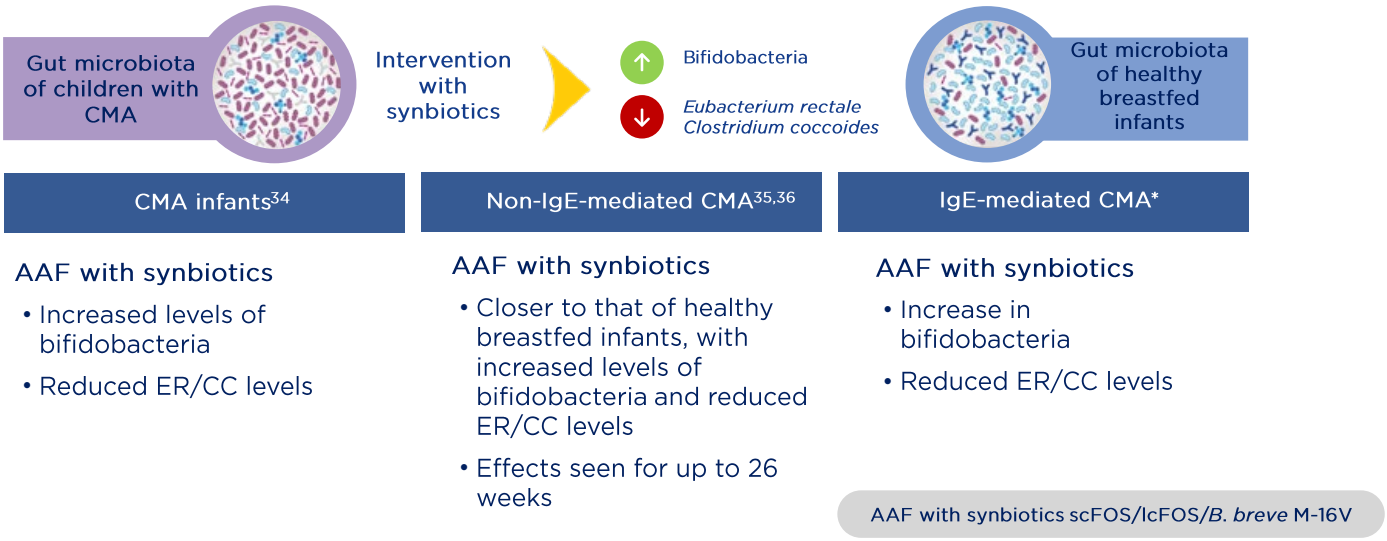

Figure 1: Three randomised controlled trials demonstrated that synbiotics rebalance the gut microbiota in infants with cow's milk allergy and bring it closer to that of healthy breastfed infants.

*PRESTO, unpublished data.

AAF: amino acid-based formula; *B. breve* M-16V: *Bifidobacterium breve* M-16V; CC: *Clostridium coccoides*; CMA: cow's milk allergy; ER: *Eubacterium rectale*; lcFOS: long-chain fructo-oligosaccharides; scFOS: short-chain fructo-oligosaccharides.

Adapted from Burks et al.,[34] Candy et al.,[35] and Fox et al.[36]

In the panel discussion, Prof Papadopoulos asked how synbiotics compare to human milk oligosaccharides, and whether synbiotics are safe. Prof Knol answered that the evidence on synthetic human milk oligosaccharides in infant formula is still scarce but he highlighted that synbiotics are a different concept compared with synthetic human milk oligosaccharides because they also contain live bacteria on top of the substrates these bacteria need. In this way, synbiotics bring live microbes into the ecosystem and feed them, leading to SCFA production and growth of bifidobacteria. This whole system approach supports the developing microbiome in early life so that it is similar to that of breastfed infants. The specific synbiotic mixture that has been well studied has proven safety in healthy infants, allergic infants, and in preterm infants.

In conclusion, a healthy gut microbiota is important for the development of the immune system and defence against infections. Human breast milk helps this process because it contains antimicrobials/antibodies, as well as substrates for beneficial bacteria, which steer healthy maturation. Synbiotics mimic human breast milk by combining prebiotics and probiotics to target the gut microbiota dysbiosis seen in infants with CMA. Three RCT have confirmed that a specific synbiotic mixture is able to rebalance the gut microbiota in infants with CMA, bringing it closer in composition, and also in activity, to that of healthy breastfed infants.

Effect of Synbiotics on Tolerance Development in Infants with IgE-Mediated Cow's Milk Allergy

Professor Kirsten Beyer

Cow's milk is one of the most common food allergens in early life. The prevalence of IgE-mediated CMA ranges from 0.5% to 3.0% at 12 months of age.[38-40] The most common symptoms are immediate type reactions, which are usually IgE-mediated, followed by worsening of atopic eczema, and non-IgE-mediated GI diseases.

In the first 3 years of life, cow's milk is a typical trigger of food-induced anaphylaxis, as shown in the European Anaphylaxis Registry of 1,970 children.[41] Natural tolerance development

is common. Data from the EuroPrevall birth cohort of >12,000 children from nine European countries followed-up over 3 years showed that approximately 70% of children with CMA outgrew their allergy within 1 year.[40] These data confirm previous results from another cohort study conducted 30 years ago.[42] However, other studies generate a heterogeneous picture, with slower outgrowth of CMA.[43,44] This mixed picture may be explained by different study populations and methodologies; for example, by retrospective collection of data or lack of follow-up at fixed intervals with oral food challenges. Infants with IgE-mediated CMA usually experience later outgrowth compared with those with non-IgE-mediated CMA; this was shown in three studies conducted in children with each type of CMA.[40,42,45]

The ongoing PRESTO trial has been investigating the effect of an AAF with synbiotics on the natural history of CMA. Conducted in 18 study sites across six countries, the trial was designed to evaluate the development of cow's milk tolerance and safety of an AAF including synbiotics in infants with IgE-mediated CMA. The trial enrolled 169 infants aged 0–13 months with IgE-mediated CMA confirmed through an oral food challenge or anaphylactic history. Infants with cow's milk protein-induced anaphylaxis or multiple food allergies were also included.

Participants were randomly allocated to an AAF with synbiotics (scFOS/lcFOS/*B. breve* M-16V; n=89) or an AAF without synbiotics (n=80) for 12 months. After the 12-month intervention, the children were followed-up for a further 24 months for a total of 36 months. Follow-up will continue for another 36 months (i.e., a total of 6 years). The primary outcome was the proportion of subjects developing tolerance to cow's milk after 12 months of intervention, as measured by a double-blind, placebo-controlled food challenge. Secondary outcomes of the trial will be development of tolerance to cow's milk at 24 and 36 months. Other endpoints include stool outcomes, adverse events, concomitant medication use, and growth.

Results have been reported after 12-month intervention and a further 12 months of follow-up (i.e., a total of 24 months).[46] Looking at the study population overall, after 12 months of intervention, 49% of the children had outgrown their CMA (Figure 2). At the 24-month follow-up, 62% had outgrown the disease.[46]

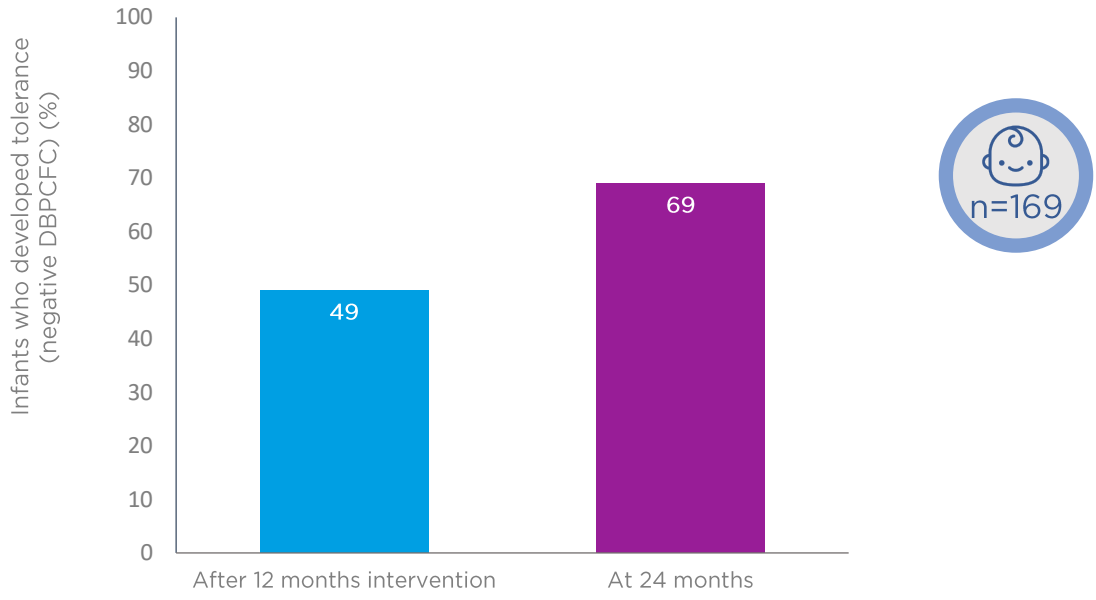

Figure 2: Approximately 50% of children receiving amino acid formula with or without synbiotics develop cow's milk tolerance after 12-month intervention.

DBPCFC: double-blind, placebo-controlled food challenge.

Adapted from Chatchatee et al.[46]

Outgrowth was good in both study groups at 12 and 24 months, with no significant differences in the proportions developing tolerance. After 12 months of the intervention, 45% of the group allocated to an AAF with synbiotics developed cow's milk tolerance compared with 52% of the group receiving an AAF without synbiotics (p=0.401). At 24 months, 64% of the test group had developed cow's milk tolerance compared with 59% of the control group (p=0.530).[46]

Outgrowth in the PRESTO study population was in line with three studies that previously reported on the proportion of infants with IgE-mediated CMA who achieved natural tolerance to cow's milk after 12-18 months, which ranged from 38% to 57%.[40,42,46] An additional study by Canani et al.[47] examined an extensively hydrolysed casein formula (eHCF), with or without the probiotic *Lactobacillus rhamnosus* GG. Prof Beyer said that looking at these results against those of PRESTO gives the impression that children in PRESTO developed cow's milk tolerance faster than those receiving an eHCF with or without *L. rhamnosus* GG.

Regarding safety, during the 12-month PRESTO intervention, significantly fewer infants in the test group (8.8%) required hospitalisation as a result of serious adverse events categorised as infections, compared with the control group (20.2%; p=0.036) (Figure 3).[46]

During the panel discussion, a query was raised about the possible reasons why outgrowth of CMA in the PRESTO trial was similar in infants who received synbiotics compared with those who did not. Prof Beyer said that the researchers were very happy to see the fantastic tolerance development, which was similar to observations in other studies, particularly because previous experts have questioned whether an AAF may not induce tolerance. Contrary to that view, the AAF used in PRESTO gave similar, and possibly faster, tolerance development to an eHCF in another study.[47] Further, Prof Beyer said the investigators were slightly disappointed that there was no difference between the two groups, and the result remains unexplained. She is very curious to see the long-term effects on other food allergies and other atopic diseases from this trial.

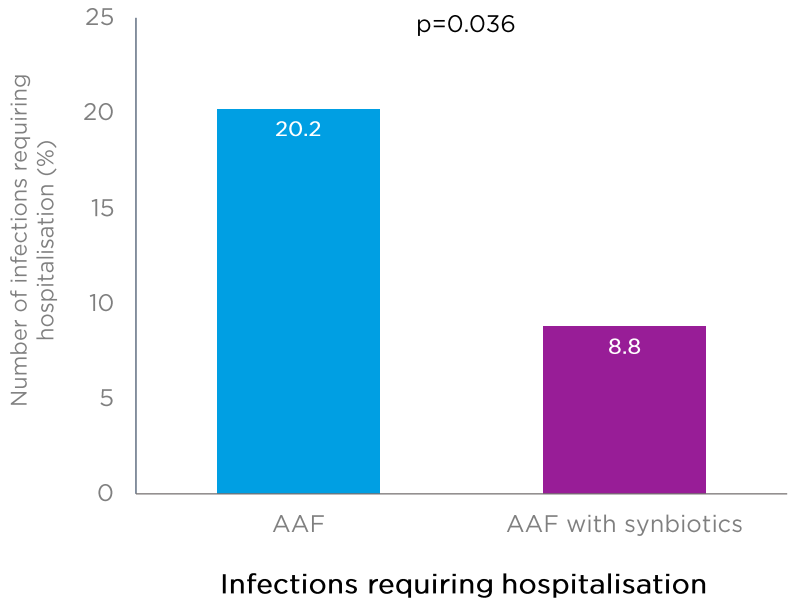

Figure 3: An amino acid-based formula with synbiotics led to reduced infections requiring hospitalisation in the PRESTO trial.

AAF: amino acid-based formula.

Adapted from Chatchatee et al.[46]

Prof Papadopoulos queried whether the development of cow's milk tolerance in 70% of children is perhaps the maximum that can be achieved. He also noted that children in PRESTO might have been exposed to some milk allergen, which could have influenced the results. Regarding the possibility of a threshold of tolerance development, Prof Beyer said this was unlikely and was hopeful that the remaining 30% of children would outgrow their allergy. She added that there is still the potential for a difference between study groups at the 6-year follow-up. As for exposure to cow's milk, Prof Beyer highlighted that children with CMA often receive small amounts as a result of contamination in bakery products and other foods, so this was unlikely to have had an impact on the results of the trial.

Prof Beyer was asked whether her views on how to manage CMA have changed with the results of PRESTO. She replied that the recommendation should still be to eliminate cow's milk from the diet. Following the PRESTO trial, the replacement formula could be an AAF, given that it demonstrated similar tolerance compared to an eHCF.

In conclusion, in the PRESTO trial, approximately one-half of children with IgE-mediated CMA developed oral tolerance to cow's milk within 12 months, and an additional 13% outgrew their allergy within 24 months. The tolerance development observed in PRESTO is in line with the literature. In addition, children in PRESTO who received an AAF with synbiotics required fewer hospitalisations as a result of infection compared with those allocated to an AAF without synbiotics.[46]

Clinical Benefits of Synbiotics Beyond the Dietary Management of Infants with Cow's Milk Allergy

Professor Anna Nowak-Wegrzyn

Simple infections can have a considerable impact on the lives of young children. During the first 3 years of life, children experience multiple infections, particularly of the upper respiratory tract.[48,49] While those infections are rarely a cause of mortality in well-developed industrialised countries, they do have a detrimental impact on overall childhood health, hospitalisation rates, and quality of life. In addition, the increased use of healthcare resources, parental work absenteeism, and secondary infections in parents and siblings creates an economic burden for society.[50,51]

Infants and young children are predisposed to infections because of the immaturity of the immune system at birth. Maturation occurs during the first 3–5 years of life. The Copenhagen Prospective Study on Asthma in Childhood 2000 (COPSAC 2000) reported a median of 14 infectious episodes in otherwise healthy children aged 0–3 years, with a large and unexplained variation in individual susceptibility (the number of infections ranged from 2 to 43).[50] Respiratory tract infections were the most common, with fewer cases of fever and GI infections. Infections peaked at around 1 year of age and declined thereafter. The study also found that 25% of infections were treated with antibiotics, with higher rates of use for ear infections and respiratory tract infections. The most frequently used drug was amoxicillin (59.4%), followed by penicillin (27.9%).

Infants and young children with CMA are more susceptible and more prone to recurrent otitis media (ear infection) compared to those without CMA.[52,53] A retrospective analysis of 280 infants with CMA showed that sensitisation to whey protein was associated with a 4-fold increased risk of recurrent respiratory tract infection before 2 years of age.[54]

As discussed by Prof Beyer, the PRESTO trial found that infants with IgE-mediated CMA who received an AAF with synbiotics had significantly fewer hospitalisations as a result of infection compared with those allocated to an AAF without synbiotics (8.8% versus 20.2%; p=0.036) (Figure 3).[46] This finding was consistent with previously reported data from two RCT of AAF with synbiotics (a blend of scFOS/lcFOS and *B. breve* M-16V).

The first trial, in infants with CMA, reported that the synbiotic formula led to significantly fewer infections (2% versus 18%; p=0.008) and use of systemic antibiotics (17% versus 34%; p=0.049), specifically amoxicillin (9% versus 32%; p=0.004), compared with an AAF without synbiotics.[34] The

second trial, which enrolled children with non-IgE-mediated CMA, found significantly fewer ear infections (0% versus 20%; p=0.011) and less use of anti-infectives (8.6% versus 34.4%; p=0.018) in infants fed with the synbiotic formula.[35,36] A preliminary systematic review of the three trials suggested that the synbiotic-containing AAF had a protective effect against ear infections in children with CMA and was linked with reduced antibiotic use for ear infections compared with an AAF without synbiotics.[55] This effect is of special relevance for atopic infants who are born with a predisposition to develop frequent infections as a result of the immaturity of their T helper Type 1 anti-infective responses. In summary, new generation hypoallergenic infant formulas with synbiotics mimic the immunomodulatory effects of breast milk, improve the profile of the gut microbiota, and result in fewer infections in early life.

During the panel discussion, Prof Nowak-Wegrzyn was asked to explain the differences in infection rates between breastfed and formula-fed infants and the benefits observed with synbiotics. She highlighted that breastfed infants generally have fewer upper respiratory and GI infections compared with those fed with formulas. Children with CMA are more prone to infections than healthy infants, which often leads to prescription of oral antibiotics, even for viral infections. This unnecessary use of antibiotics contributes to disturbed gut microbiota. However, there could be a remedy: an exploratory analysis in >300 infants with CMA from three trials showed the same signal, namely that an AAF with synbiotics may reduce ear infections and associated antibiotic usage compared to an AAF without synbiotics (ear infections: 0-6% with synbiotics versus 11-20% without synbiotics; medication for ear infections: 0-4% with synbiotics; 9-17% without synbiotics).[55]

Prof Nowak-Wegrzyn was asked whether she would advise parents to use synbiotics, and in which situations and at what time. She replied that very strong evidence is needed to publish a guideline, and she thinks that the point will be reached when there is sufficient data to recommend the use of synbiotics. Breastmilk continues to be the best source of nutrition for all infants, including those with CMA, and should be promoted as much as possible. If formula is indicated, she would consider recommending one with synbiotics because growth and safety are equivalent to formulas without synbiotics and the rate of infections requiring hospitalisation appears to be lower. She predicted that in future, synbiotics will be indicated in situations in which the gut microbiota may be disturbed, such as infants delivered by caesarean section, or when the mother is receiving antibiotics during pregnancy or lactation.

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Nutritional Management of Cerebral Palsy in Children

This Nestlé Health Science Online Symposium took place on 2nd October 2020

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Disclosure: Prof Gottrand has received grant/research support from Lactalis; and has served as a member of an advisory board for Nestlé Clinical Nutrition. Prof Romano has received consultancy fees from AbbVie, Nestlé, and Nutricia. Prof Marchand has participated in speakers bureau for Abbott, Nestlé, and Takeda Pharmaceutical Company.

Acknowledgements: Medical writing assistance was provided by Dr Eleanor Roberts, Beeline Science Communications, Ltd, London, UK, on behalf of EMJ.

Disclaimer: The views and opinions expressed are those of the presenters.

Support: The publication of this article was funded by Nestlé Health Science. Content was reviewed by the speakers for medical accuracy.

Citation: EMJ. 2020;5[4]:29-38.

Meeting Summary

Due to feeding and digestion difficulties, children with cerebral palsy (CP) can be at risk of malnutrition. European nutritional guidelines regarding children with neurological impairment (NI) have stressed the importance of identifying nutritional difficulties through factors beyond weight and height, such as assessment of fat mass, bone mineral density, and nutritional status. Feeding difficulties can be caused by a combination of oral- and gut-related problems, such as postural complications, swallowing difficulties, and gastro-oesophageal reflux disease (GORD). If oral feeding is too difficult, or is unsafe, a feeding tube may need to be inserted either into the stomach or jejunum. Once the feeding method is established, other considerations include ensuring energy needs are being met. These must be individually assessed because of large differences in energy needs and body composition. One way such needs can be met is through the use of formulas with adequate caloric and nutritional values; another is by using blenderised food, tailored to the individual's dietary needs and preferences. Further gastrointestinal problems include diarrhoea and constipation, which may also be helped with a blenderised food diet and/or with addition of dietary fibre to formulas. Such nutritional management of children with CP involves a multidisciplinary team of healthcare professionals, the child, and their family. During this symposium, Prof Romano, Prof Gottrand, and Prof Marchand discussed findings from their own practices, professional guidelines, and clinical studies that can aid in identifying nutritional deficiencies and managing the nutritional needs of children with CP.

Overview

CP, the most common NI in children, is caused by nonprogressive damage or malformation while the brain is developing. The manifestation of CP is heterogenous and can affect an individual's speech, motor skills, vision, memory, muscle actions, and learning abilities.[1]

While advances in supportive care, which have mainly focussed on respiratory and orthopaedic problems, have extended the life expectancy of people with CP, what also needs to be addressed is another problem experienced by patients: chronic undernutrition or malnutrition.[2] In this series of three talks, Prof Romano discussed 'Guidelines and importance of fibres in paediatric enteral nutrition [EN],' Prof Gottrand talked about the 'Importance of percutaneous endoscopic gastrostomy [PEG] and patient's energy needs: how and which formula to use,' and Prof Marchand provided practical advice on the use of blenderised food in her talk, 'Use of real food in real life.'

The Problem of Nutrition and Malnutrition in Children with Cerebral Palsy

Assessment of Nutritional Status

A recent study of 325 children with CP found that approximately 75% were underweight, 50% had dysphagia, and >40% had more than one risk factor for malnutrition.[3] These findings suggested that nutritional needs and feeding challenges of all children with CP should be assessed regularly. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Guidelines working group (ESPGHAN-WG), of which Prof Romano was the lead, has provided in-depth guidelines for evaluating and treating gastrointestinal and nutritional complications in children with NI.[4]

Assessment of undernutrition/malnutrition in a child with CP should not be based on CP-specific growth charts as, discussed Prof Gottrand, "CP children often have a low body size [both height and weight] and [a different] composition from neurologically developed children." Instead, according to the ESPGHAN-WG, signs of malnutrition include a weight for age that is ≥2 standard deviations below the mean and fat mass measurements (triceps skin fold, arm muscle area) below the 10th percentile. Assessment should also include measurement of bone mineral density and micronutrients, as well as being vigilant of other warning signs of malnutrition, such as skin problems associated with prolonged sitting or lying, poor peripheral circulation, and failure to thrive.[4]

While the digestive system of a child with CP may be physiologically normal, receiving adequate nutrition can be impacted by many different factors. These include oral problems, such as swallowing difficulties, drooling, and dental abnormalities; postural difficulties caused by pain, spasticity, and hip luxation; and digestive problems, such as GORD, oesophageal motility, vomiting, aerophagia, delayed gastric emptying, and constipation.[2,4,5] GORD is a particular problem in children with CP that can be associated with poor feeding.[5,6] Investigations for this condition include endoscopy, intraluminal impedance and pH-metry, and biopsy. If these are difficult to perform, the ESPGHAN-WG have suggested an initial trial of proton pump inhibitors (PPI).[4]

Feeding difficulties can also arise as a result of factors such as a lack of appetite, cognitive problems, depression, and medication side effects.[2,4,5] "This is important to keep in mind," discussed Prof Gottrand, "because some of these factors have therapeutic options. Correcting dental abnormalities, treating pain, treating depression, limiting drug side effects, or treating GORD could all be tried rather than going directly to tube feeding or a gastrostomy."

Energy Needs of Children with Cerebral Palsy

One goal of adequate nutrition is to meet a person's energy needs. The ESPGHAN-WG recommend using dietary reference standards for typically developing children to gauge the number of calories needed by a child with CP.[4] Approximately 70–75% of energy expenditure, explained Prof Gottrand, "is explained by basal metabolic rate, which is strongly influenced by body size and composition, especially fat-free mass." As such, it was also noted that reference standards may overestimate energy needs because of the low weight and height of many children with CP and variation in muscle tone.[4]

Energy requirements may also vary owing to level of disability and ambulatory ability. For example, energy expenditure was examined in one study with regard to the Gross Motor Function Classification System (GMFCS), which ranges from Level I, where someone can walk without restriction, to Level V, where self-mobility is severely limited.[7] In the study, in those who could ambulate to any degree (Levels I–IV), a significantly higher amount of energy was needed to perform the same walking task as someone without any disability when each level was compared with the one below.[8]

Interventions for Nutritional Deficiencies in Children with Cerebral Palsy

For children with CP, oral feeding can be considered as long as it is safe, nutritionally sufficient, stress-free, and takes no longer than 3 hours a day. However, dental problems, postural difficulties, and orthopaedic issues that can contribute to oral feeding hazards mean that while oral nutritional intake may be adequate, it may not be considered safe on account of dangerous occurrences, such as pulmonary aspiration (Figure 1).[4]

Prof Romano discussed how there are some very basic ways to help a child with CP gain adequate nutrition, such as making sure their seating posture when eating is optimal, providing food of a consistency that is easy to swallow, and making sure the caloric density and fibre content of meals is adequate.[4]

Importance of Dietary Fibre

Prof Romano focussed on the beneficial effects of dietary fibre. Sources of fibre in enteral formulas include those that are soluble, such as pectin and guar, which are fermented to short-chain fatty acids by colonic bacteria and provide fuel for large intestine endothelial cells, and those that are insoluble, such as soy polysaccharide, which increases faecal weight and colonic peristalsis.[9,10] Another use of dietary fibre is for prebiotic needs thanks to its resistance to gastric activity, enzyme hydrolysis, and gastrointestinal absorption. Fibres can also stimulate growth and activity of 'healthy' intestinal bacteria, such as bifidobacteria.[11]

Recent EN guidelines from the European Society for Clinical Nutrition and Metabolism (ESPEN) recommended, in adult and paediatric populations, fibre-containing enteral feeds, particularly when a person is experiencing diarrhoea or constipation.[12] In support of this, a study in which a standard enteral diet was enriched with 20 g/L of partially hydrolysed guar gum found that diarrhoea occurrence was significantly reduced compared with the standard diet alone.[13] Further, two meta-analyses in adult populations confirmed a reduction in diarrhoea or constipation incidence when an enteral formula included fibre.[10,14]

During the discussion, Prof Romano suggested that soluble fibre was preferred to alleviate diarrhoea, although "it is viscous and can be difficult [to administer] when using a gastrostomy and tube feeding." Prof Gottrand highlighted: "As many CP children don't move, constipation is frequent and hard to treat and they need high quantities of laxatives." He discussed how the amount of fibre included even in fibre-enriched formulas may be too low to correct gastrointestinal problems, including constipation, and thus would need enhancing. As such, all speakers agreed that ≥10 g/day of extra fibre may be needed, especially in nonambulatory children, although studies have not been carried out to ascertain optimal fibre intake.

Enteral Nutrition

For those unable to gain enough nutrition through oral feeding, EN via a PEG tube may be required to supplement or replace the oral method.[4] If problems such as GORD-related aspiration, refractory vomiting, retching, and bloating occur with PEG feeding, the ESPGHAN-WG suggested using jejunal feeding.[4]

Prof Gottrand stressed "the crucial importance in taking parents/caregivers on board when discussing the process of gastrostomy feeding." The decision whether to use EN has to be made by assessing the needs of not only the child, but also their parents/caregivers and the family as a whole (Figure 2). For the child, important considerations include both physical aspects of EN, including benefits and complications, social aspects of how PEG feeding fits in with their daily routine, and quality of life.

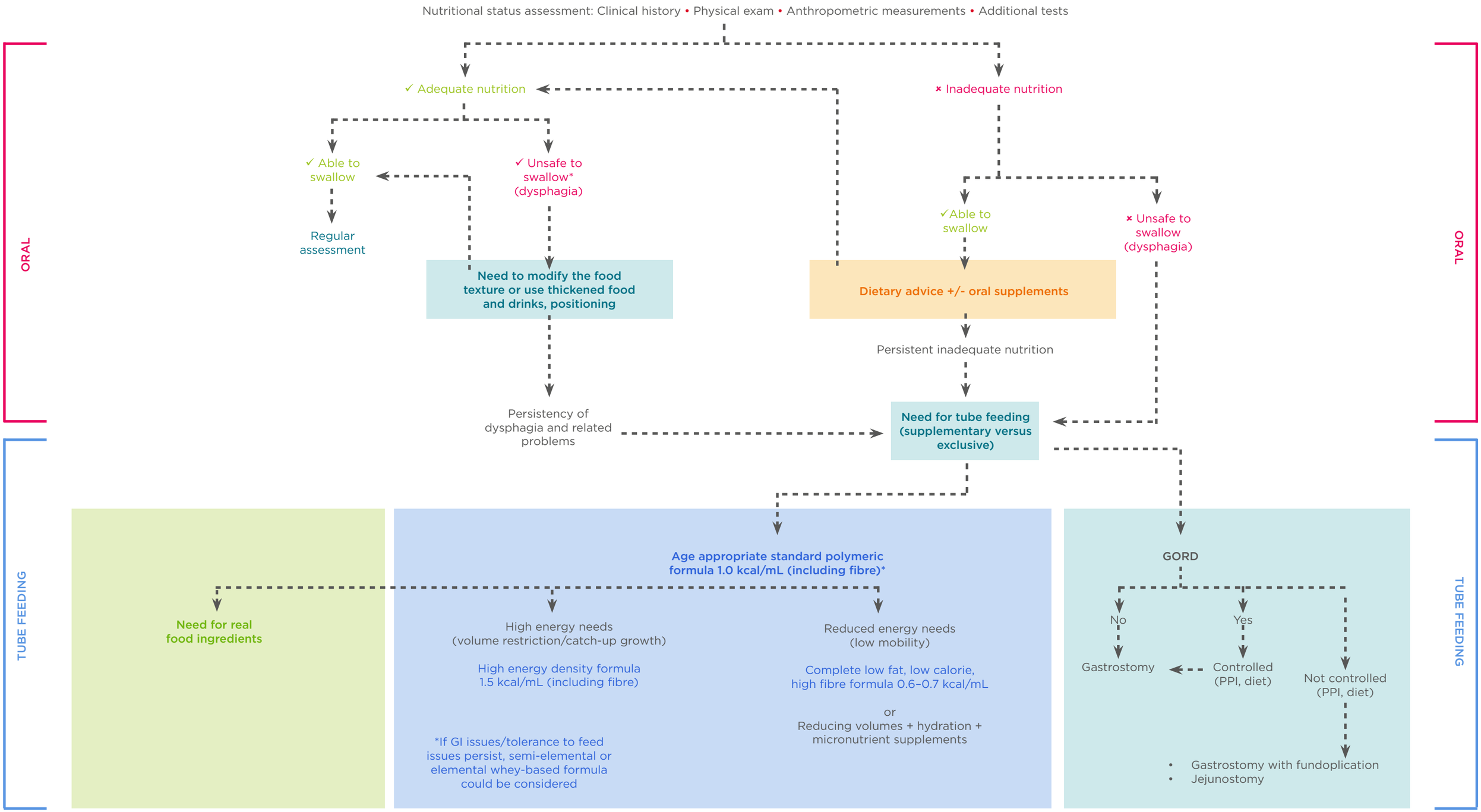

Figure 1: Nutritional management of children with neurological impairment.

Unsafe swallowing is defined as a history of aspiration pneumonia (antibiotics or hospital admission for chest infection) and objective evidence of aspiration or penetration on contrast videofluoroscopy.

GORD: gastro-oesophageal reflux; GI: gastrointestinal; PHGG: partially hydrolysed guar gum; PPI: proton pump inhibitor.

Adapted from Romano et al.[4]

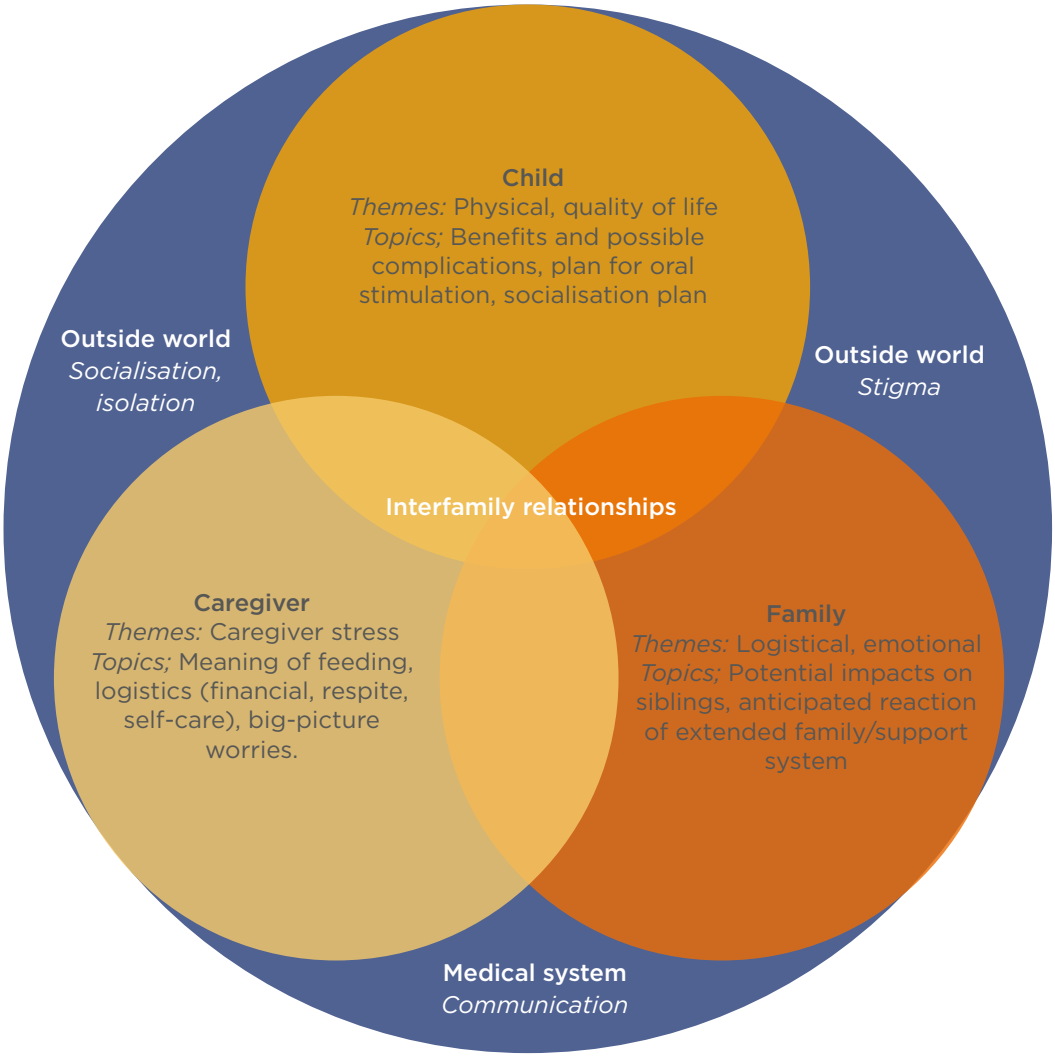

Figure 2: Themes and topics to be considered during discussions regarding placement of a gastrostomy tube.
Adapted from Nelson et al.[15]

For the parent/caregiver, there are challenges regarding how PEG feeding is carried out, logistical considerations including financial needs, and awareness of how much stress PEG feeding may cause. For the family, considerations may include how PEG feeding fits into mealtimes, as well as emotional aspects such as impact on siblings, and whether wider family support is available. Considerations outside of the family unit include stigma, socialisation, and communication within the medical system.[15]

The primary benefit of EN is that nutritional status and growth can be improved.[16,17] For example, in a study of 368 children/young adults (age: 1 month–25 years old) tube fed via PEG, a significant weight and height catch-up was observed over a median follow-up of 2.4 years.[17] Other benefits include alleviation of oral feeding problems, such as coughing and choking, and improvement in quality of life for the parents/caregivers.[18]

The type of EN used depends on a number of factors, such as the child's age, their energy requirements, and the mode of enteral access. It may, according to Prof Gottrand, be given as a combination of daytime bolus feeds and nocturnal continuous feeds, especially in children with high-caloric needs or poor tolerance to high volumes of food in one sitting. EN will often be in the form of a commercially available formula, and the ESPGHAN-WG have suggested that a standard (1.0 kilocalorie [kcal]/mL) polymeric

age-appropriate formula including fibre is adequate for most children past their first year. In those who cannot tolerate a high volume of food, a high-energy density formula (1.5 kcal/mL) may be required. The ESPGHAN-WG also recommended at least yearly checking of protein and micronutrient intake compared to dietary reference standards, with supplementation if needed.[4]

During EN feeding, some assessments need to be regularly carried out, such as monitoring of body weight and fat mass. Additional nutrition may be needed for 'catch-up growth'; however, once this is achieved there is a risk of over-feeding, leading to obesity.[4] In the discussion, it was asked whether over-feeding can be diminished by reducing formula volume administered or by diluting it. "Though this is possible," Prof Romano remarked, "the risk is that [reducing the amount] won't cover the volume needs of these children as many don't experience thirst so are chronically under-watered." Dilution also has risks, he continued, as this could "reduce the micronutrient and fibre intake, which is often low as energy need is low."

In children who are immobile, low energy needs suggest a low-fat, low-calorie, high-fibre, and micronutrient-replete formula may be needed.[4] Assessment of this in one study of 14 mostly immobile children with CP (age: 10 months–11 years old) who had received a PEG found that after 6 months, adequate nutritional status and growth, without excess weight, was achieved despite the fact that the formula was 50% of the estimated average energy requirement for children of their respective ages.[19] In the discussion, it was highlighted that as formula volume may be very low in those with low energy needs, it is important to maintain fluid levels because, according to Prof Gottrand, problems such as kidney stones could arise. Prof Marchand pointed out that a practical way to overcome this is to include fluid rinses after feeding and medication administration. Another problem related to these low calorie EN formulas that arose during the discussion was that they are unfortunately not available in some countries and/or are not reimbursable via the healthcare system.

As with oral feeding, GORD can be a problem when feeding via a PEG (Figure 1). In a study of 326 children/young adults with a newly-situated gastrostomy (age: 1 month–25 years old), 12% experienced new incidences of GORD and it was exacerbated in 25% of the 74% who already experienced it.[20] ESPGHAN-WG suggestions for treating EN-related GORD include thickening the formula, using a whey-based formula, and using a PPI.[4] Another solution is antireflux surgery, as was required by 16% of those with GORD in the above study.[20] During the discussion, when asked whether different nutritional therapies were needed for those who had undergone similar surgery, Prof Gottrand said that while there were few comparison studies, in his experience there is no clinical advantage of any one formula type.

Blenderised Tube Feeding

Before 1970, reported Prof Marchand, tube feeds often consisted of blenderised food. While commercial formulas took over for a period of time, there has been a growing trend among families and dietitians to revert to blenderised food. A recent survey of blenderised tube feeding (BTF) found over one-half of 54 adult patients using EN used BTF.[21] In another survey, nearly 90% of 125 children who required long-term EN were administered BTF for a mean of 71% of their total daily nutritional intake.[22] Additionally, a survey of registered dietitians found that 58% used BTF, with most agreeing the experience was positive for the family, child, and the clinical practice.[23]

The use of BTF depends on a team of people including the child's physician, to assess medical suitability; a registered dietitian, to help devise recipes using advanced nutrition analysis software; and caregivers, to evaluate daily aspects of BTF administration. These people need to work together, stressed Prof Marchand, to assess these aspects both prior to initiation and throughout.[24] Two recent examinations of how best to start with BTF highlighted a variety of aspects for consideration.[24,25] The patient should be medically stable on EN with a mature gastrostomy tube of at least size 12 French, they should have the ability to tolerate bolus feeding, and medical and family support should be adequate for both initiation and maintenance, with the right equipment and ability for food preparation and storage.[25] Prof Marchand also pointed out some contraindications, such as in babies <6 months old, patients in intensive

care, individuals who are fed via a nasogastric or nasojejunal tube, and in children who require continuous feeds.

With home-made blenderised foods, it is important that the medical team evaluate the child's nutritional needs so that personalised recipes can be provided. This is important because a study of 433 parents of children who were tube fed found that 49.5% of those who used blenderised food did so without any professional guidance.[26]

Blenderised meals usually include a liquid base, such as milk or formula; a protein source; grains; fruits; vegetables; and oil, with micronutrients, sodium, and water added as required.[27] Prof Marchand advised that recipes also need to account for local and seasonal food availability, variety, and limiting potential environmental toxins, such as mercury in fish and arsenic in rice. Once prepared, blenderised food must be refrigerated, but must be warmed prior to administration. BTF is delivered as a bolus and should be administered in <2 hours. During the discussion, Prof Marchand highlighted how BTF is not exclusive and can be combined with night-time feeds with formula. Caregivers need to be taught many other aspects of blenderised food preparation as well as composition, including portion size, correct measuring techniques, how to read food labels for nutritional value, and how to clean and store food preparation utensils.[24]

The advantages of blenderised food, reported Prof Marchand, include that it can be tailored to individual nutritional and micronutritional needs, such as specific food allergies and intolerances. It may also be perceived as being more natural than commercial formulas, and "gives the parents a feeling of normalcy," helping them feel nurturing and in control of their child's nutrition.[26] Feeding and digestion-related advantages include an increase in oral intake and a decrease in gagging,[28] improvements in diarrhoea and vomiting,[29] and microbiome diversity.[30] Prof Marchand also highlighted how, in her experience, "the use of a real food blend is also the most effective way to address reflux, better than an elemental or semi-elemental diet." Additionally, blended food may be less expensive than commercial formula if the latter is not covered by a person's healthcare provider.[25]

To examine the use of blenderised food, the 6-month 'BLEND' study included 20 children (age: 1–16 years old) with a PEG who, at baseline, used commercial formula for 75% of their calorie intake. Caregivers were provided with instructions for prescription formulas taking into account their child's calorie, liquid, portion, and sodium needs. It was noted in this study that 50% more calories than usual feeds were needed to maintain a stable BMI with blenderised food, although micronutrient intake was similar or better. Mean energy intake, which at the beginning of the study was 74 kcal/kg, increased to 111 kcal/kg with blenderised feeds.[30]

After 6 months, there was an increase in the number of children with a triceps skin fold measurement >5th percentile, from 76% to 82%, and in the percentage of participants who consumed something by mouth (67% versus 80%). Vomiting more than once a week decreased (76% versus 53%) and gagging/retching also decreased (82% versus 47%). There was a decrease in the number of children needing antacid medication (88% versus 76%), and an increase in those who required a stool softener (24% versus 29%). Significant changes were found in microbial diversity and richness in stool samples (Figure 3). Importantly, the study also found that most caregivers (94%) agreed that BTF was successful and that their child appeared in better health and was happier. All caregivers said they would recommend BTF.[30]

As examples of successes with blenderised food, Prof Marchand described her first experiences, both of which involved 4-year-old children with a PEG whose families had tried multiple different formulas and feeding regimens. For the first child, who had a very short bowel, the main problem was severe diarrhoea and bacterial overgrowth. The child was able to feed orally, though intake was limited. As a result of BTF, the child's diarrhoea decreased; there was less bacterial overgrowth; decreased need for antibiotics; improved nutritional status; and, eventually, she was weaned off the PEG and all blenderised food was administered orally. For her second patient, who did not feed orally, the main problems were inability to gain weight, vomiting, and GORD. The latter was unresolved with prokinetics and PPI, therefore antireflux surgery was considered. Following the use of BTF, the child gained weight, the vomiting resolved, and surgery was avoided.

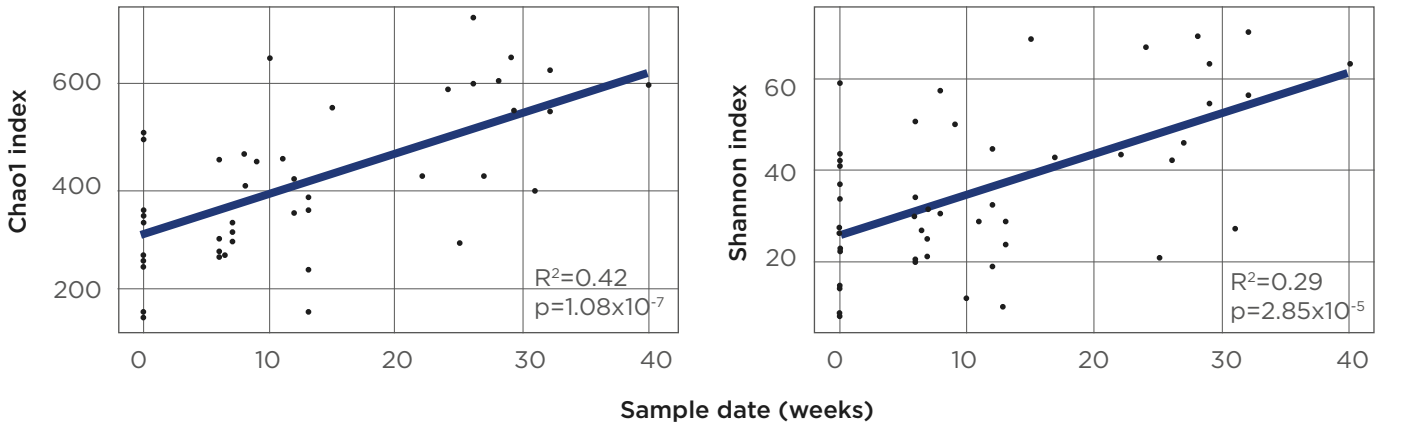

Figure 3: α diversity (Chao1 index) and microbial richness (Shannon index) of microbiota from stool samples with the BLEND diet.

α diversity indices were calculated using data rarefied to 50,000 reads per sample. Statistical significance of increased species diversity and richness was calculated using linear regression with $p<0.05$ considered significant.

Adapted from Gallagher et al.[30]

While there are several advantages to blenderised food, Prof Marchand also discussed some disadvantages. For example, the composition of blenderised food is not standardised; a larger volume is required; it requires bolus feeding, often with a syringe that can quickly wear out; tubes can become obstructed; it can be time consuming; and it may cost more as it will not be covered by medical insurance. Prof Marchand highlighted commercially available, real food-based formula as a convenient and efficient alternative. Prepared food may be more liable to contamination than commercially prepared EN; however, a comparison between blenderised food and standard polymeric formula and a BTF made using commercial baby food found no difference in bacteria content after being left for 2–4 hours.[31] It remains very important to stress to parents that blenderised food should only be used in bolus or short-time infusion to avoid the risk of bacterial contamination.

In conclusion, Prof Marchand stressed that "we, as healthcare professionals, need to keep an open mind and see the benefits [of BTF]. We need to support parents in their quest to provide their child with the best and provide them with guidance to do it in a safe manner with adequate nutritional follow-up."

Conclusion

The high prevalence of undernutrition, growth impairment, poor body mass density, and micronutrient deficiencies in children with CP means ongoing assessment and monitoring of nutritional requirements and needs are vital. These require a multidisciplinary team, including a gastroenterologist, neurologist, dietitian, specialist nurse, and family members.

If needed, EN can provide the nutrients crucial to maintain a child's health. This may require supplementation with fibre and can consist of both commercially available EN formulas, including real food-based formula and blenderised food, according to individual needs and tolerances.

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TIM-3 is expressed on immune cells and myeloid cells, so targeting it provides potential to activate immune response and suppress leukemic cell proliferation.[1,2]

- Challenges facing current standard-of-care therapies include poor overall survival, limited duration of response, and singular mechanistic action[3-6]
- Additional approaches are needed to improve treatment options and patient outcomes[7-9]

Novartis is researching the role of TIM-3 in HR-MDS and AML.

See both sides at DualTIM3.com

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

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Interview

Prof Ilona Kickbusch

Chair of the Advisory Group of the Global Health Programme at the Graduate Institute of International and Development Studies, Geneva, Switzerland
Independent Global Health Consultant, Kickbusch Health Consult, Brienz, Switzerland

Q1

In your impressive, nearly four-decade career in global health, particularly with the World Health Organization (WHO), how have strategies and attitudes about global health responsibilities changed over that time?

In that period, we moved from an understanding of international health to global health, which implied the strong global interconnectedness between countries and peoples with regard to health. It was also a period when many more actors and influential people became part of the global health universe next to the WHO, new organisations like the Joint United Nations Programme on HIV/AIDS (UNAIDS); the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM) and the Global Alliance for Vaccines and Immunisation (GAVI); many more civil society organisations; and of course, the Bill and Melinda Gates Foundation. The range of private sector actors has increased as well, think of the tech industry. Attitudes and approaches have changed over time, from a strong commitment to primary healthcare, to strong vertical programmes, back to strong universal health coverage commitments, for example. There are many more strategic alliances, also owing to the Sustainable Development Goals (SDG), and now of course, following the coronavirus disease (COVID-19) pandemic, the need to work together across countries and sectors is becoming more evident by the day. Global health is global, it is not a new word for health in developing countries according to a development aid model.

Q2

You have been involved in both health promotion directly to the public, and in developing health policy and governance programmes. Which strategy, in your experience, has had a greater impact on global health?

I don't think you can juxtapose this. People are social actors, and they need to be able to have the knowledge to take care of themselves and their loved ones, but this does not happen in a vacuum. That's why the strategy of 'empowerment' has been so important: what other mechanisms need to be in place so that people's voice on their health needs is heard. But the best health promotion programme cannot replace decisive action on the social determinants of health; it is inequality, poverty, and racism that kills. This means that strategies to address health inequalities are critical; this also includes action on the commercial determinants of health, for example, protecting children from marketing harmful to their health. The role of the state is always central: think of taxes on unhealthy goods,

labelling of unhealthy products, and regulations for safe working conditions and housing. The list is long. At the global level, this can mean looking at trade strategies or agreeing to international rules, such as the Framework Convention on Tobacco Control.

Q3

You have championed the 'Health in All Policies' strategy at the WHO. In this time of economic difficulty and environmental urgency, how can this role for health action be emphasised in governmental and organisational planning?

There is no better time. Health is central to all other 'crisis' agendas; COVID-19 shows us there can be no health security without social security, and no economic development without a healthy population. Economic and health development go hand in hand. Global Health has long argued this. The same is true for the environmental agenda; think of the local level where a policy to increase cycling will lead to more exercise as well as better air quality. Now in 'corona times' it can also be safer to cycle than to sit in public transport. We speak of cobenefits: by working together, other sectors benefit as does health. Any Health in All Policies agenda must work from that premise.

Q4

You now work as an independent global health advisor. After moving away from organisations such as the WHO, do you think that the political side of these organisations helps or hinders their efforts to improve global health?

Health is always political and it would be negligent for any health strategy to neglect that. Politicians have interests, as do other groups in society, and countries have national interests, which they bring to the international level. That is why it needs international bodies, such as the WHO, where these interests are negotiated; we have called this health diplomacy. What we are seeing right now is different from that: it is using health as a political tool in a geopolitical stand-off. This type of politicisation is something we have not had before, and it is incredibly dangerous for the health of the world. On the other hand, a large majority of countries has agreed to come together to jointly address the development, production, and distribution of COVID-19 vaccines. Within about 4 months, the world has created a new mechanism of sharing — not perfect, but quite extraordinary.

Q5

There seems to have been an evolution of your strategies to improve global health over your career: from community health promotion, to health literacy and individual education, to health diplomacy and policy approaches. Has your opinion of effective strategies changed over time, or do you think there is a role for each of these strategies (community promotion, individual education, political policy) in global health?

No, my opinion has not changed; for me this is consistent, and just always draws on different aspects of the Ottawa Charter that I helped launch and develop. The five areas of the Charter,

"COVID-19 shows us there can be no health security without social security, and no economic development without a healthy population."

policy, environments, community action, personal skills, and changing the healthcare system, are interdependent. Over time, new dimensions and movements have emerged to address these different aspects. Take the fact that the Ottawa Charter was the first WHO document to mention the need for ecological changes; I wrote one of the first texts that brought health promotion in line with the ecological challenges (Good Planets are Hard to Find). For me personally as a political scientist, looking more closely at global governance became important following my experiences at the WHO.

Q6 Your education background is not in healthcare. Do you think more clinical professionals should be involved in global health, or is an understanding of economics and politics valuable in the global domain?

Global Health is clearly totally interdisciplinary, but not all understandings of global health programmes follow this dictum, to their detriment. We have seen in the Ebola Crisis how anthropologists were added as an afterthought, and now with COVID-19, the role of political and economic analysis is suddenly considered relevant in order to explain the very different responses by countries and political leaders. Right now, you cannot understand global health without an analysis of geopolitics. People's health behaviour, their health beliefs, and their motivations in turn cannot be understood without the social sciences; you cannot develop a strategy to address opposition to vaccines without behavioural science.

Q7 In recent years, you have leveraged your reputation to advocate for increased female representation in global health. What has been your experience of barriers to women's representation in global health decision-making, and how do you think it can be improved in the future?

I was one of the first senior women in the WHO and I was already a feminist when I joined the organisation. I had been active in the women's health movement in Germany. Experiences were tough but I made my way; I am a resilient person. But I also had very supportive mentors; actually, all of them men. When I joined the WHO, I was different along various counts: nonmedical, young, and female, so it was also not always clear what people were reacting to. A few years ago, it really hit me that I was still one of the few senior women in global health, so I decided to do something about it. Luckily, this was picked up by many younger women and came at the right time, when a wave of dissatisfaction about being excluded from global health leadership had started to emerge. This is great, and it now also includes a strong call for voices from the Global South and structurally excluded groups in the Global North; it's all about decolonising global health. That's what the young people's movements are doing now, and there are already many fantastic next-generation global health professionals and researchers changing the field.

Q8 You developed the settings-based approach to health promotion in the WHO, creating initiatives for healthy cities, healthy schools, and healthy workplaces. Do you think that this settings focus is the best strategy for addressing the COVID-19 pandemic, or is it more effective to focus on the behaviour and education of individuals?

Again, this is not 'either or': all good public health is a mix of strategies that support one another. The settings approach built on the understanding of creating supportive and enabling environments for people's health behaviours. This also applies during COVID-19; we have the combination of things people need to do (social distancing, hand washing, wearing masks) and the settings related to it, as expressed in the Japanese strategy of avoiding the three C's: closed spaces, crowded places, and close-contact settings. You then need people in control of the settings to act responsibly (in restaurants for example), and in some cases you need regulations like wearing masks in public transport. It is always the combination of strategies that wins out through reinforcement.

Q9 Global health inequalities seem heavily entrenched in economic and geographic situations, including in the current COVID-19 pandemic. Can there be any global strategy for addressing COVID-19 that could be successful across these different contexts, or should the principles of global health be applied in more targeted ways?

"...a large majority of countries has agreed to come together to jointly address the development, production, and distribution of COVID-19 vaccines. Within about 4 months, the world has created a new mechanism of sharing — not perfect, but quite extraordinary."

There exists a global strategy to address inequalities: the SDG clearly set the direction of "leave no one behind." There are many ways to do this in various contexts; right now, we are experiencing a rebound in global poverty and increased difficulties for disadvantaged groups. In many cases, women are again paying the price. What we realise is that we need to invest billions, if not trillions, in global health, and that the usual financing through development aid is totally insufficient. Last years' United Nations General Assembly (UNGA) called for significant investments in universal health coverage; they have been complemented by the investments that are required for pandemic preparedness. Still, these billions are 'small' amounts compared with the trillions of losses experienced economically during the pandemic. We need a rethink of global health financing, and a significant boost in the financing of the WHO if we are to move forward.

Q10

In your role as Director of the Global Health Programme at the Graduate Institute of International and Development Studies, Geneva, Switzerland, what do you hope your students will achieve in their global health careers?

After 10 years, I have passed on the leadership of the Global Health centre to two excellent codirectors. I remain chair of the advisory group and still work within the centre. My goal has always been that students understand the political dimensions of global health; that's why founding this centre at a Graduate Institute of International and Development Studies was so critical. Politics is about power. We need these students coming from very different disciplines learning to work together during their studies and bringing this analytical mind-frame to wherever they might go on to work.

Q11

Your editorial article 'Health promotion 4.0' published last year provided a fascinating look at parallels between the foundational attitudes of the Ottawa Charter (1986) and the recent Montreal Declaration for a Responsible Development of Artificial Intelligence (2018). How do you see the role of global health advocates changing as digital landscapes alter health, wellbeing, health data, and medical interventions?

This, in my view, is one of the most important areas of health promotion in the next 10 years. I don't think the role of global health advocates changes; it's the areas of advocacy that they need to address that are changing. The digital transformation of our world, of health systems, and of health and well-being is progressing at a rapid speed. It is essential that health promotion on the one hand sees the potential and the opportunities, but at the same time analyses carefully what the dangers could be, in my article I call it "the dark side." I hope that the next WHO global conference on health promotion in 2021 will take these issues forward and outline approaches based on equity, human rights, and empowerment.

Primer on the Pathogenesis of Severe COVID-19: Part One

EDITOR'S PICK

Our Editor's Pick for this *EMJ* flagship issue is a two-part review by Walsh that discusses the pathogenesis of coronavirus disease (COVID-19) by firstly providing a breakdown of the complex cellular host–viral interactions, before discussing the key mediators of specific pathogenicity. With the search for a vaccine remaining top of the agenda worldwide, an increased understanding of the pathophysiology of this disease is essential in guiding the therapeutic approaches being put in place to stem the spread of this pandemic. We hope that you enjoy reading this timely review.

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Disclosure: The author has declared no conflicts of interest.

Received: 23.06.20

Accepted: 27.08.20

Keywords: Cluster of differentiation 147 (CD147), coronavirus disease (COVID-19), NRLP3 Inflammasome, pneumonia, protein kinase R, severe acute respiratory syndrome (SARS), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

Citation: EMJ. 2020;DOI/10.33590/emj/20-00158

Abstract

In Part One of this exploration of the pathogenesis of coronavirus disease (COVID-19), the author will evaluate the viral and cellular immunological basis for the condition. The virus demonstrates a remarkable capability not just to evade, but to exploit host immune characteristics to perpetuate viral replication. In this regard, severe acute respiratory syndrome (SARS)/severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) disables most antiviral mechanisms, including the early interferon response, and avoids detection to permit unimpeded viral multiplication. Consequently, antigen-presenting cells fail to adequately stimulate the T-cell receptor. As a consequence, T-cell p53 remains highly expressed, which in turn disables an adequate effector T-cell response.

Replicating SARS-CoV-2 double-strand RNA robustly activates protein kinase R (PKR)/PKR-like endoplasmic reticulum kinase (PERK). While the virus is grossly invulnerable to its antiviral effects, PKR is crucial for effecting the cytokine milieu in COVID-19. PERK is a component of the unfolded protein response, which eventuates in autophagy. SARS virions use double-membrane vesicles and adapt PERK signalling not only to avoid autophagy, but to facilitate replication. Viral activation of PKR/PERK is mutually exclusive to NLRP3 stimulation. The NLRP3 pathway elaborates IL-1β. This is chiefly a feature of paediatric SARS/SARS-CoV-2 cases. The difficulties encountered in predicting outcome and forging effective therapeutics speaks to the breadth of complexity of the immunopathogenesis of this virus.

INTRODUCTION

Severe and fatal severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection is characterised by chiefly pulmonary manifestations. Clinically, pneumonias have been subdivided into specific phenotypes: a spectrum from patchy ground-glass opacification to the oedematous lung with atypical acute respiratory distress syndrome features.[1] Bilateral diffuse alveolar damage with cellular fibromyxoid exudates and desquamation of pneumocytes with hyaline membrane formation are pathologically apparent.[2] For the majority of patients (varying by age and other factors), the condition has been mild. It appears that the causative virus, SARS-CoV-2, evolved into two major genomic types (L and S types), with a roughly 70:30 split.[3] Although the L-type is likely phenotypically more aggressive, variability in host response is clearly a major determinant of outcome. Studies of SARS-CoV-2 have been limited by the novel nature of the virus. Nonetheless, valuable insight may be drawn from existing studies on the biology and pathogenesis of the SARS virus because of the significant sequence homology.[4]

VIRUS-SPECIFIC CHARACTERISTICS: ESTABLISHING INFECTION

Extrapolating from SARS-CoV virology, initial investigations implied that SARS-CoV-2 used angiotensin-converting enzyme 2 (ACE2) for cellular entry.[5] ACE2 expression is predominantly, but not limited to, pulmonary tissue (Type 2 pneumocytes and ciliated airway epithelial cells), with expression of SARS virus previously reported in extrapulmonary ACE2+ tissues.[6-9] SARS spike proteins (S-proteins) were found to bind robustly to ACE2.[10,11] Recent reports suggest that the nature of SARS-CoV-2 affinity for ACE2 is 10-times more avid than that of SARS. TMPRSS2, a serine protease, is used for S-protein priming within cells, which is essential for viral spread and (especially pulmonary) pathogenesis in the infected host (cathepsins B and L may also be used but are inessential for this purpose).[5,12] A further portal of entry is cell surface expression of cluster of differentiation 147 (CD147), also known as basigin or extracellular matrix metalloproteinase inducer (EMMPRIN).[13-15]

Of the large coronaviral genome, the open reading frames 1a and 1b (ORF1a and 1b), encoding the viral replicase, comprise two-thirds. The replicase includes multispanning transmembrane proteins that physically anchor the replication/transcription complex to the intracellular membranes. Within the cytoplasm, double-membrane vesicles (DMV) branch off from the rough endoplasmic reticulum (ER), reminiscent of autophagosomes.[16] The coronavirus endonuclease, which resides in the replication complex, prevents simultaneous activation of double-strand RNA (dsRNA) sensors melanoma differentiation-associated protein 5, 2'-5'-oligoadenylate synthetase 3, and protein kinase R (PKR). This strategy permits evasion of the host innate antiviral defenses.[17] Thus, viral kinetics are rapid in the early period (first 48 hours) post-infection.

THE IMMUNOLOGICAL RESPONSE IN SEVERE COVID-19 INFECTION

Severe SARS/SARS-CoV-2 is suggestive of an apparent biphasic (dysregulated) immune response.[18] A weak or absent interferon Type 1 ([IFN-1] i.e., IFNα and IFNβ) response during the early phase of SARS-CoV-2 infection plays an important role in permitting viral replication within nasopharyngeal cells and pneumocytes.[19,20] It is apparent that virion nonstructural protein 1 within infected cells can suppress host gene expression (including IFN-1), promote host messenger RNA (mRNA) degradation, and inhibit host protein translation.[21] Furthermore, the SARS coronavirus papain-like protease induces inhibition of the production of IFN-1 and proinflammatory cytokines in toll-like receptor 3 (TLR3), retinoic acid-inducible gene 1, and TLR7 signalling pathways, thus disabling detection mechanisms in the endosome and in the cytoplasm.[22-26]

Indeed, some investigators could find no or only modest evidence of IFN response to SARS infection.[27,28] However, fatal/severe SARS in humans was accompanied by a late but robust and persistent expression of IFN-1, especially from plasmacytoid dendritic cells. Concurrent expression of IFN-1 and IFN-stimulated genes appear to preclude adequate T-cell and antibody responses;[18,29-31] this was demonstrated in SARS-infected IFNαβ receptor knockout ($IFNAR^{-/-}$)

mice, who exhibited only mild to moderate weight loss and clinical disease. These mice demonstrated minimal alveolar oedema and increased peribronchiolar/perivascular immune cell infiltration, which resolved with viral clearance by 10 days post-infection. By contrast, BALB/c mice with intact IFNAR exhibited a six-fold increase in (mainly) inflammatory monocyte-macrophages by Day 3 post-infection, which is too late for the peak of viral replication. This was then abrogated in the absence of IFN-1.[32]

Augmented T-cell apoptosis in SARS infection impedes T-cell response and engenders a relative lymphopaenia.[33] Normally, IL-2 promotes the differentiation of effector T cells in the presence of antigen-specific T-cell receptor (TCR) stimulation. TCR engagement by peptide-bound major histocompatability complex (MHC) molecules on antigen-presenting cells (APC) changes the topology of the TCR, inducing the formation of microclusters at the T cell–APC interface, named the immunological synapse. This arrangement helps to improve T-cell antigen recognition.[34]

However, in SARS, antigen-specific TCR expression is weakened by an early hyporesponsiveness of APC, especially in dendritic cells. SARS-CoV does not upregulate the expression of CD83, CD86, MHC Class I, or MHC Class II molecules on immature dendritic cells.[35] Also, the late surge in IFNβ alters histone modifications in the IL-2 promoter to retain the locus in an inaccessible configuration, thereby curbing the T-cell response.[36] Any elevation in IL-2 expression, secondary to immune monocyte-macrophage stimulation of naïve T cells, induces a sustained increase in p53 protein expression in the T cell. Downmodulation of p53 is necessary for antigen-specific responses of naïve and antigen-primed peripheral T cells and T-cell clones. This prevents proliferative TCR signalling, critical for antigen-specific CD4(+) T-cell responses, despite adequate signalling through the IL-2 receptor.[37] It is this failure to recruit CD4+ T cells to the lungs (and not CD8+ T cells) that adversely affects viral clearance. The absence of the CD4+ T-cell response leads to enhanced interstitial pneumonitis.[18] Furthermore, respiratory tract memory CD4+ T cells, in conjunction with IFNγ, would provide a more robust intermediate-term defence against reinfection.[38] The latter may account for the inconsistent nature of postinfective antibody production.

T cells can attenuate cytokine storms by suppressing the immune response.[39] It is the impairment of the regulatory mechanisms of T cells in a cytokine-rich milieu that fosters the development of the cytokine storm.[40]

In view of the abortive nature of T-cell infection by SARS-CoV-2, it is unlikely that this, per se, makes a significant contribution to the observed lymphopenia in coronavirus disease (COVID-19) infections.[41]

DISEASE-SPECIFIC IMMUNOLOGY

The Role of Protein Kinase R

While the impairment of host gene expression undoubtedly plays a part in infected epithelial cell apoptosis, much of the apoptosis appears to be secondary to host PKR expression, which is engaged as a stratagem to detect replicating dsRNA. PKR induces phosphorylation of elongation initiation factor 2α (eIF2α) which, in turn, blocks protein synthesis through translation of mRNA. The phosphorylated eIF2α also encodes for antiviral factors and mediates the integrated stress response.[41] The blockade of protein synthesis results in the decrease or prevention of viral replication, and may result in apoptosis.[42] PKR can also induce apoptosis independently of eIF2α phosphorylation, by activation of the FAS-associated death domain (FADD)/caspase-8/caspase-3, and caspase-9 apoptotic protease-activating factor-1 (APAF-1) pathways.[43-46] In spite of a significant burden of PKR expression, SARS-CoV is not susceptible to its antiviral activities. Indeed, rather than inhibiting PKR activation, translation of SARS-CoV mRNA proceeds despite eIF2α phosphorylation.[42]

PKR increases IFN1 expression via an eIF2α-independent mechanism by regulating IFNα/β mRNA stability.[41,47] As well as contributing to the development of interstitial pneumonia, this spike in IFN1 creates an autoamplifying loop, given that IFN1 is known to enhance PKR expression.[42] Activation of PKR by dsRNA has been shown in several cell types, including airway epithelial cells, to result in phosphorylation of IκB and therefore activation of NF-κB.[48-51] p38 mitogen-activated protein kinase (MAPK) expression requires PKR.[52,53] dsRNA induction of TNF-α requires PKR activation, but IL-1β induction follows a PKR-independent pathway.[54] IL-6, IL-8, and Regulated upon Activation, normal T-cell expressed and

presumably secreted (RANTES), among others, are promoted by PKR expression.[42] Exuberant induction of PKR is the substantial driving force behind the cytokine-rich milieu observed in SARS/SARS-CoV-2 infection (Figure 1).

Note should be made here that levels of PKR may be already elevated chronically in individuals by virtue of a string of host factors including obesity, ageing, diabetes, congestive heart failure, cancer, and genetic factors (e.g., ApoE4, the most prevalent risk factor for Alzeihmer's disease), all of which have been found to yield worse outcomes in SARS-CoV-2 infections.[55,56]

The Role of NLRP3 Inflammasome (Paediatrics)

Paediatric SARS patients were demonstrated to have markedly elevated circulating IL-1β levels. This suggests selective activation of a caspase-1-dependent pathway. Notably, only mild/minimal activation of IL-6 and TNF-α was noted in paediatric populations.[57] However, as indicated above, IL-1β is independent of the PKR pathway. Rather, IL-1β is a caspase cleavage product of the nucleotide-binding domain and leucine-rich repeat and NLRP3 pathway.[58] It appears that the SARS-CoV ORF8b robustly activates the NLRP3 inflammasome by providing a potent signal 2, required for activation. Note that inflammasome activity requires signals to effect cleavage: signal 1 is a priming signal and signal 2 is an activation signal. Mechanistically, ORF8b interacts directly with the leucine-rich repeat domain of NLRP3 and localises with NLRP3 and apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) in cytosolic dot-like structures. ORF8b triggers cell death consistent with pyroptotic cell death in macrophages; while in those cells lacking NLRP3, accumulating ORF8b cytosolic aggregates cause ER stress, mitochondrial dysfunction, and caspase-independent cell death.[59] Likewise, SARS-CoV ORF3a protein activates the NLRP3 inflammasome by promoting TRAF3-dependent ubiquitination of ASC.[60]

It has been shown that PKR can suppress the NLRP3 inflammasome activation by modulating the microtubular cytoskeleton.[61,62] It is evident, therefore, that a key mediator of the differential clinical presentation apparent between the paediatric population (who almost universally experience a mild or at worst moderate infection) and older adults (especially those with the aforementioned comorbidities, who are more predisposed to severe illness with COVID-19) is whether or not the NLRP3 pathway is activated in preference to a mutually exclusive pathway involving PKR overexpression.

The relatively rare occurrence of the Kawasaki-like syndrome as part of the spectrum of paediatric presentation with COVID-19, known as paediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 infection in Europe or multisystem inflammatory syndrome in children in the USA, bears the hallmarks of an autoinflammatory syndrome/vasculitis, whose pathogenesis owes to IL-1β overexpression.[63,64]

The Role of Protein Kinase R Endoplasmic Reticulum Kinase

PERK is also elaborated as a consequence of dsRNA presence within the cytoplasm. PERK is a sensor in the unfolded protein response pathway.[65] PERK is further activated by the SARS-CoV 3a protein. As well as causing eIF2α phosphorylation, it stimulates expression of ER molecular chaperones such as glucose-regulated protein 78 (GRP78) and GRP94.[66] Increased transcriptional activation and biosynthesis of ER chaperones would enhance folding of the 3a and other viral proteins in the ER lumen. ER-associated degradation (ERAD) will normally delete an increased load of unfolded and misfolded proteins within the ER.[67] This requires activation of the Inositol-requiring enzyme-1/X-box binding protein-1 and activating transcription factor 6 pathways. However, by activating PERK only, and not these ERAD components, the SARS-CoV 3a protein is able to enhance folding of common viral structural proteins S, E, M, and N, while avoiding activation of ERAD (which would be detrimental to virion assembly and likely trigger autophagy-dependent cell death).[68]

SARS-CoV must traffic across the ER membrane, thereby forming structures called double-membrane vesicles (DMV), which are thought to provide the necessary platform for the viral replication process while avoiding immune detection.[69] DMV, which originate from the ER membrane, contain nonstructural transmembrane proteins (nsp)3 and nsp4 and viral dsRNA,[70,71] but lack markers typical for the ER Golgi intermediate compartment or Golgi.[72]

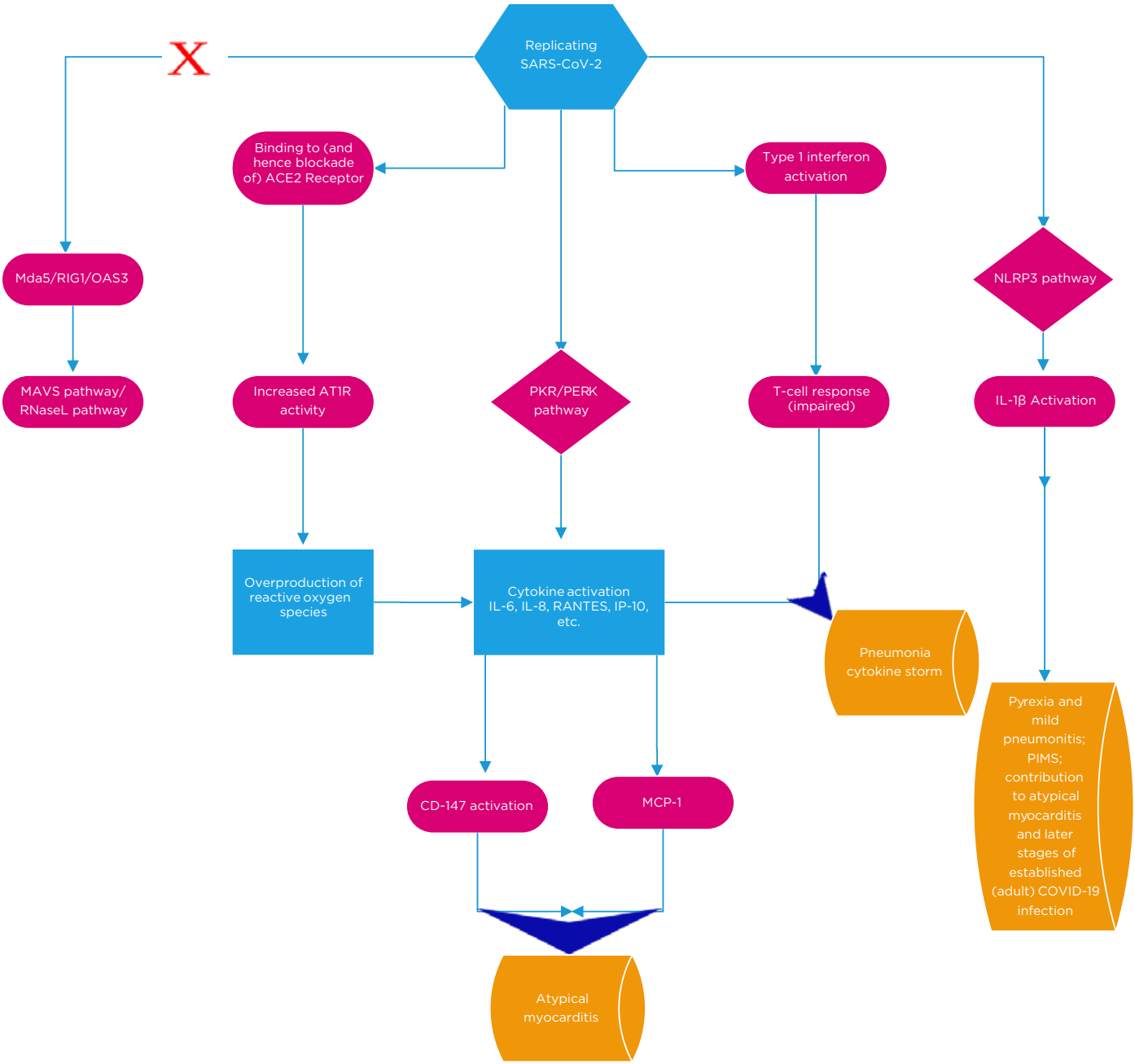

Figure 1: Overview of the basic cellular mechanisms contributing to coronavirus disease (COVID-19) pathogenesis.

Viral S protein binding to ACE2 receptors results in preferential activation of AT1R. The net consequence of this is a local overexpression of reactive oxygen species. In adults, the PKR/PERK pathways are preferentially activated to the exclusion of other antiviral pathways. This leads to the upsurge incytokine production. Subsequent consequences of this include activation of CD147 and MCP1, which contribute to atypical myocarditis. SARS CoV-2 initially disables the Type 1 interferon response but, subsequent to peak viraemia, Type 1 interferons are produced to excess. This impairs T cell response. The pro oxidant milieu in conjunction with the cytokine excess and blunted T cell response culminate in COVID-19 pneumonia and cytokine storm (across a spectrum of clinical severity). The NLRP3 pathway appears to be active early in paediatric infections to the exclusion of PKR/PERK (and at a later stage in adult disease). The clinical consequences include a relatively milder pneumonitis and pyrexia and rarely, in extreme circumstances, PIMS. It also contributes to the later stages of infection in adults.

ACE2: angiotensin converting enzyme 2; AT1R: angiotensin 1 receptor; *RIG1*: retinoic acid inducible gene I; MAVS: mitochondrial antiviral signaling protein; MCP1: monocyte chemoattractant protein 1; Mda5: melanoma differentiation associated protein 5; OAS3: oligoadenylate synthetase; PERK: protein kinase R endoplasmic reticulum kinase; PIMS: paediatric multisystem inflammatory syndrome; PKR: protein kinase R; RANTES; regulated upon activation, normal T cell expressed and presumably secreted; RNaseL: ribonuclease L; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

DMV are coated with microtubule-associated protein light chain 3 (LC3), which is a ubiquitin-like modifier.[73] These ubiquitin-like modifiers recognise specific receptors that target associated vesicles to particular cellular locations.[71,72] LC3 can exist in in a lipidated (LC3-II) or a nonlipidated form (LC3-I) form. LC3-II is involved in fusion of autophagosomes to lysosomes/proteomes,[74] but coronavirus DMV display the nonlipidated LC3-I and thereby evade destruction.[71]

In overview, it is clear that the PKR/PERK pathway is used for propagation of the viral lifecycle and is central to the cytokine-driven pathogenesis of the disease. This acknowledgement is not an assertion that PKR/PERK is the only pathway active in severe COVID-19 in adult patients. SARS-CoV-2 is a positive-sense single-stranded RNA virus that activates PKR/PERK during the replicative process when dsRNA is present. When viral replication is reduced, the NLRP3 inflammasome can become active, particularly in later stages of infection. In addition, the NLRP3 caspase cleavage product, IL-1β, may be elaborated via noninflammasome means (e.g., during a protracted inflammatory process via release of neutrophil serine proteases).

THE CONTRIBUTION OF CD147 TO SEVERE DISEASE PATHOGENESIS

Further to the exploitation of the PKR/PERK pathway, SARS-CoV-2 may also use the inflammatory milieu as a means to further its own cell invasive/replicative potential. For instance, IL-6 has previously been demonstrated to promote expression of CD147, a key receptor for viral cellular entry.[75-77] This may be a normally adaptive process in the host since its expression may, in conjunction with cyclophilins, contribute to the recruitment of immune cells to sites of inflammation via chemokine-like activity.[78] Cyclophilins have previously been found to contribute to coronavirus pathology. Indeed, overexpression of SARS-CoV nonstructural protein 1, as well as infection with live SARS-CoV, strongly increased signalling through the calcineurin/nuclear factor of activated T cells (NFAT) pathway and enhanced the induction of IL-2. The latter is compatible with the immunopathogenicity and cytokine dysregulation observed in severe SARS cases.[79]

CD147/EMMPRIN is a widely expressed integral plasma membrane glycoprotein that is expressed at varying levels in many cell types, including haematopoietic, epithelial, and endothelial cells.[80-82] Its cell surface expression (with cyclophilin cofactors) has been associated with interactions with extracellular matrix proteins, such as matrix metalloproteins, and integrins.[83,84] While it is appreciated that IL-6 alone can be responsible for induction of thromboinflammation, it appears that CD147 overexpression is also proficient in this role, eliciting leukocyte chemotaxis and adhesion, as well as platelet activation and subsequent thrombus formation through the binding of various interaction partners.[85-87] CD147 may assist IL-6 with the intravascular expression of vitronectin, plasminogen activator inhibitor-1, and von Willebrand factor, which forms the prothrombotic phenotype in severe SARS cases.[88,89]

THE VIRAL INDUCTION OF OXIDATIVE DAMAGE

The SARS-CoV-2 spike protein binds with great avidity to the ACE2 receptor.[12] Once the protein has been cleaved by the serine protease TMPRSS2, the virus can be endocytosed, likely via micropinocytosis.[90] Another serine protease, ADAM-17, is activated upon binding of SARS-CoV to ACE2 and facilitates viral entry. In fact, knockdown of ADAM-17 by small interfering RNA severely attenuates SARS-CoV cellular entry. ADAM-17 functions as a TNF-α cleavage enzyme and thus it contributes to the presence of TNF-α in SARS pneumonia.[91] Pulmonary endothelial expression of ACE2 is lower in older adults relative to younger individuals.[92] This would appear to imply that older adults should be less susceptible to contracting the virus (at least at a cellular, biological level) and has also served as something of a paradox in that patients with chronic obstructive pulmonary disease who actively smoke have higher levels of ACE2, but have had relatively low mortality compared to those with cardiovascular disease who tend to have relatively low expression of ACE2.[93,94] Angiotensin II levels have been demonstrably high in patients with COVID-19, thus apparently vindicating the theory that viral sequestration of ACE2 has a biochemical consequence

for the host.[95] In spite of this, patients who contract COVID-19 are not rendered overtly hypertensive. Moreover, the renin–angiotensin system is activated in almost any physiological stress scenario.[96]

In the absence of ACE2, signalling via the angiotensin I receptor is enhanced, apparently contributing to lung injury and pulmonary oedema in SARS.[97,98] Angiotensin II binding to the angiotensin I receptor mediates its adverse effect on the lung through various subtypes of NADPH oxidase to produce reactive oxygen species.[99] Furthermore, NADPH oxidase enhances phosphorylation, and hence activation, of PKR.[100] The specific pathogenic contribution of ACE2 sequestration to COVID-19 pneumonia has been reviewed elsewhere.[101]

Haem oxygenase-1 (HO-1) is a stress-inducible, anti-inflammatory, and cytoprotective enzyme expressed in most cell types in organisms. Under several stress stimuli, HO-1 expression and activity is upregulated to catalyse the rate-limiting enzymatic step of haem degradation into carbon monoxide, free iron, and biliverdin.[102] Besides its effects on cell metabolism, HO-1 is also capable of modulating host innate and adaptive immune response to sepsis, transplantation, and autoimmunity, and prevents oxidative damage associated with inflammation. HO-1 can exert a significant antiviral activity against a wide variety of viruses.[103] Its activation decreases the migration of polymorphonuclear leukocytes to the lung. This may, in certain circumstances, reduce oxidative tissue damage.[104] Crucially, it has been demonstrated to inhibit dendritic cell activation and immunogenicity.[105] Failure to recruit functional respiratory dendritic cells to the lungs has been identified as a key defect, permitting the pathogenesis of SARS-CoV-induced pneumonia.[106,107] This may be pertinent in the early stages of SARS/SARS-CoV-2 pneumonia.

In myeloid cells, HO-1 forms a complex with interferon regulatory transcription factor 3 (IRF3), which is required for IRF3 target genes and alters responses in infected cells.[106] In fact, HO-1-deficient macrophages will show reduced expression of IFN-β and IRF3. SARS coronavirus papain-like protease inhibits IRF3 activation at a step after phosphorylation, which is dependent on the deubiquitination activity of papain-like protease.[109] In later stages of the infection, caveolin-1 overexpression in lung epithelial cells will competitively inhibit HO-1 (caveolin-1 will be discussed further in Part Two).[110]

FUTURE DIRECTIONS: USING PATHOGENESIS TO BUILD DRUG PLATFORMS

The PKR/PERK pathway is the pathogenic motive force behind the severity of COVID-19. The NLRP3 inflammasome fuels an alternative, mutually exclusive, and apparently more benign condition. It follows that blockade of the PKR/PERK pathway would be distinctly advantageous to the host. While the expectation would be that it would not prevent infection, the nature of the infection would be much less severe.

To date, several pharmacological inhibitors of PKR have been investigated with varying degrees of adequacy. It is clear that identification of an inhibitor of PKR with good drug properties is an urgent necessity. There are some useful articles assessing this specific topic.[111-114] N-acetylcysteine has been demonstrated to partially remediate the apoptotic consequence of PKR activation and oxidative stress.[100] Furthermore, N-acetylcysteine has been demonstrated to alleviate the cytokine overproduction that occurs in alveolar Type II cells in the context of other respiratory viral infections such as influenza A and B and respiratory syncytial virus.[115] N-acetylcysteine accomplishes this through inhibition of NF-κB translocation to the nucleus and phosphorylation of p38 MAPK, both of which are part of a stress pathway induced by PKR to promote IL-6, IL-8, and monocyte chemoattractant protein 1 overproduction.[115-118]

Pharmacological inhibitors of CD147 are also being developed for heterogenous indications.[119-120] There may also be some rationale behind use of the humanised anti-CD147 IgG2 monoclonal antibody, meplazumab, which has been licensed as an orphan drug by the U.S. Food and Drug Administration (FDA) for treatment of malaria to counter not just viral entry into cells, but also the deleterious effects induced by CD147 in severe COVID-19.[121] However, pharmacological evaluation is beyond the scope of this paper.

CONCLUSION

In conclusion, the author offers a rational breakdown of the cellular host–viral interaction in COVID-19. A substantial component of this analysis is extrapolated from the laboratory work already done in the field of SARS-CoV and other coronaviral infection/pneumonia. In Part Two, the author will probe the key mediators of specific systemic pathogenicity in COVID-19. It is the author's strong recommendation that the details presented herewith are made subject to scrutiny in detailed animal studies involving SARS-CoV-2. Furthermore, the investigation of the platforms for drug treatment require accelerated human trials to establish efficacy and to curb the ongoing toll of lives lost and economic damage caused by this pandemic.

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Prescribing Information: LIBTAYO (cemiplimab) 350mg concentrate for solution for infusion **Please refer to Summary of Product Characteristics (SPC) prior to use.** **Presentation:** Each vial contains 350mg of cemiplimab in 7ml of solution. **Indication:** LIBTAYO as monotherapy is indicated for the treatment of adult patients with metastatic or locally advanced cutaneous squamous cell carcinoma (mCSCC or laCSCC) who are not candidates for curative surgery or curative radiation. **Dosage and Administration:** Treatment must be initiated and supervised by physicians experienced in the treatment of cancer. LIBTAYO is administered by intravenous infusion over 30 minutes through an intravenous line containing a sterile, non-pyrogenic, low-protein binding, in-line or add-on filter (0.2 micron to 5 micron pore size). Other medicinal products should not be co-administered through the same infusion line. ***Recommended dose:*** The recommended dose of LIBTAYO is 350 mg, every 3 weeks (Q3W). Treatment may be continued until disease progression or unacceptable toxicity. ***Dose modifications:*** No dose reductions are recommended. Dosing delay or discontinuation may be required based on individual safety and tolerability. Recommended modifications to manage adverse reactions are provided in Table 1 of the SPC. **Special Populations:** ***Paediatric (<18 years):*** Safety and efficacy has not been established. ***Elderly:*** No dose adjustment is recommended. ***Renal impairment:*** No dose adjustment is recommended, however there are limited data for LIBTAYO in patients with severe renal impairment (CLcr15-29ml/min). ***Hepatic impairment:*** No dose adjustment is recommended for patients with mild hepatic impairment. LIBTAYO has not been studied in patients with moderate or severe hepatic impairment. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients. **Precautions and Warnings:** ***Traceability:*** To improve traceability of biological medicinal products, the name and the batch number of the administered product should be clearly recorded. ***Immune-related adverse reactions (IRARs):*** IRARs may involve any organ system. Most IRARs initially manifest during treatment however, they can occur after discontinuation of cemiplimab. IRARs affecting more than one body system can occur simultaneously, such as myositis and myocarditis or myasthenia gravis, in patients treated with cemiplimab or other PD-1/PD-L1 inhibitors. Patients treated with cemiplimab should be monitored for signs and symptoms of IRARs. IRARs should be managed with cemiplimab treatment modifications, hormone replacement therapy (if clinically indicated), and corticosteroids. For suspected IRARs, patients should be evaluated to confirm an IRAR and to exclude other possible causes, including infection. Depending upon the severity of the adverse reaction, cemiplimab should be withheld or permanently discontinued. ***Immune-related pneumonitis:*** defined as requiring use of corticosteroids with no clear alternate aetiology, including fatal cases, has been observed. Patients should be monitored for signs and symptoms of pneumonitis and causes other than immune related pneumonitis should be ruled out. Patients with suspected pneumonitis should be evaluated with radiographic imaging as indicated based on clinical evaluation and managed with cemiplimab treatment modifications and corticosteroids. ***Immune-related diarrhoea or colitis:*** defined as requiring use of corticosteroids with no clear alternate aetiology, has been observed. Patients should be monitored for signs and symptoms of diarrhoea or colitis and managed with cemiplimab treatment modifications, anti-diarrhoeal agents, and corticosteroids. ***Immune-related hepatitis:*** defined as requiring use of corticosteroids with no clear alternate aetiology, including fatal cases, have been observed. Patients should be monitored for abnormal liver tests prior to and periodically during treatment as indicated based on clinical evaluation and managed with cemiplimab treatment modifications and corticosteroids. ***Immune-related endocrinopathies:*** defined as treatment-emergent endocrinopathies with no clear alternate aetiology, have been observed. ***Thyroid disorders (Hypothyroidism/Hyperthyroidism):*** Thyroid disorders can occur at any time during the treatment. Patients should be monitored for changes in thyroid function at the start of treatment and periodically during the treatment as indicated based on clinical evaluation. Patients should be managed with hormone replacement therapy (if indicated) and cemiplimab treatment modifications. Hyperthyroidism should be managed according to standard medical practice. ***Hypophysitis:*** Immune-related hypophysitis has been observed. Patients should be monitored for signs and symptoms of hypophysitis and managed with cemiplimab treatment modifications, corticosteroids and hormone replacement, as clinically indicated. ***Adrenal insufficiency:*** Patients should be monitored for signs and symptoms of adrenal insufficiency during and after treatment and managed with cemiplimab treatment modifications, corticosteroids and hormone replacement, as clinically indicated. ***Type 1 Diabetes mellitus:*** Immune-related type 1 diabetes mellitus, including diabetic ketoacidosis, has been observed. Patients should be monitored for hyperglycaemia and signs and symptoms of diabetes as indicated based on clinical evaluation and managed with oral anti-hyperglycaemics or insulin and cemiplimab treatment modifications. ***Immune-related skin adverse reactions:*** defined as requiring use of systemic corticosteroids with no clear alternate aetiology, including severe cutaneous adverse reactions (SCARs), such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) (some cases with fatal outcome), and other skin reactions such as rash, erythema multiforme, pemphigoid, have been reported in association with cemiplimab treatment. Patients should be monitored for evidence of suspected severe skin reactions and exclude other causes. Patients should be managed with cemiplimab treatment modifications and corticosteroids. For symptoms or signs of SJS or TEN, refer the patient for specialised care for assessment and treatment and manage patient with treatment modifications. Cases of SJS, fatal TEN and stomatitis occurred following 1 dose of cemiplimab in patients with prior exposure to idelalisib, who were participating in a clinical trial evaluating cemiplimab in Non-Hodgkins Lymphoma (NHL), and who had recent exposure to sulfa containing antibiotics. Patients should be managed with cemiplimab treatment modifications and corticosteroids as described above. ***Immune-related nephritis:*** defined as requiring use of corticosteroids with no clear alternate aetiology, has been observed in patients receiving cemiplimab. Monitor patients for changes in renal function. Patients should be managed with cemiplimab treatment modifications and corticosteroids. ***Other IRARs:*** Other fatal and life-threatening IRARs have been observed in patients receiving cemiplimab including paraneoplastic encephalomyelitis, meningitis and myositis. Evaluate suspected IRARs to exclude other causes. Patients should be monitored for signs and symptoms of IRARs and managed with cemiplimab treatment modifications and corticosteroids as clinically indicated. Solid organ transplant rejection has been reported in the post-marketing setting in patients treated with PD-1 inhibitors. Treatment with cemiplimab may increase the risk of rejection in solid organ transplant recipients. The benefit of treatment with cemiplimab versus the risk of possible organ rejection should be considered in these patients. Cases of graft versus-host disease have been reported in the post-marketing setting in patients treated with other PD-1/PD-L1 inhibitors in association with allogeneic hematopoietic stem cell transplant. ***Infusion-related reactions:*** Cemiplimab can cause severe or life-threatening infusion-related reactions. Patients should be monitored for signs and symptoms of infusion-related reactions and managed with cemiplimab treatment modifications and corticosteroids. Cemiplimab should be interrupted or the rate of infusion slowed for mild or moderate infusion-related reactions. The infusion should be stopped and cemiplimab should be permanently discontinued for severe (Grade 3) or life-threatening (Grade 4) infusion-related reactions. ***Patients excluded from clinical studies:*** Patients that had active infections or that were immunocompromised were not included in the main study. ***Fertility, Pregnancy and Lactation:*** No clinical data available on the possible effects of cemiplimab on fertility. No effects observed in fertility study on cynomolgus monkeys. Women of childbearing potential should use effective contraception during treatment with cemiplimab and for at least 4 months after the last dose of cemiplimab. Cemiplimab, as an IG4, has the potential to be transmitted across the placenta from the mother to the developing foetus. Cemiplimab is not recommended during pregnancy and in women of childbearing potential not using effective contraception unless the clinical benefit outweighs the potential risk. It is unknown whether cemiplimab is secreted in human milk. If a lactating woman chooses to be treated with cemiplimab, she should be instructed not to breastfeed while being treated with cemiplimab and for at least 4 months after the last dose. ***Interactions:*** No pharmacokinetic drug-drug interaction studies have been conducted with cemiplimab. The use of systemic corticosteroids or immunosuppressants before starting cemiplimab, except for physiological doses of systemic corticosteroid (≤10 mg/day prednisone or equivalent), should be avoided because of their potential interference with the pharmacodynamic activity and efficacy of cemiplimab. However, systemic corticosteroids or other immunosuppressants can be used after starting cemiplimab to treat IRARs. **Adverse Reactions:** ***Very common:*** Diarrhoea, Fatigue, Pruritus and Rash. ***Common:*** Alanine aminotransferase increased, Arthralgia, Arthritis, Aspartate aminotransferase increased, Blood alkaline phosphatase increased, Blood creatinine increased, Hepatitis, Hyperthyroidism, Hypothyroidism, Infusion related reaction, Musculoskeletal pain, Pneumonitis, Stomatitis, Dyspnoea. ***Uncommon:*** Adrenal insufficiency, Central nervous system inflammation, Chronic inflammatory demyelinating polyradiculoneuropathy, Encephalitis, Guillain-Barre syndrome, Hypophysitis, Immune thrombocytopenic purpura, Keratitis, Meningitis, Muscular weakness, Myasthenia gravis, Myocarditis, Nephritis, Neuropathy peripheral, Paraneoplastic encephalomyelitis, Pericarditis, Polymyalgia rheumatica, Sjogren's syndrome, Thyroiditis, Type 1 diabetes mellitus, Vasculitis. ***Rare:*** Myositis. ***Not Known:*** Solid organ transplant rejection **UK List price:** £4650 per vial. **Legal Category:** POM. **Marketing Authorisation Number:** EU/1/19/1376/001. **Marketing Authorisation Holder:** Regeneron Ireland U.C., Europa House, Harcourt Centre, Harcourt Street, Dublin 2, Ireland. **For more information please contact:** Sanofi, 410 Thames Valley Park Drive, Reading, Berkshire, RG6 1PT, UK. uk-medicalinformation@sanofi.com **Date of preparation:** August 2020.

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store. Adverse events should also be reported to the Sanofi Drug Safety department on Tel: 0800 0902314.

Alternatively, send via email to UK-drugsafety@sanofi.com

Conditional approval: LIBTAYO has been authorised under a 'conditional approval' scheme. This means that further evidence on this medicinal product is awaited.

References: 1. LIBTAYO (cemiplimab) Summary of Product Characteristics. Regeneron Pharmaceuticals, Inc. **2.** KEYTRUDA (pembrolizumab) Summary of Product Characteristics. Merck Sharp & Dohme Limited. **3.** OPDIVO (nivolumab) Summary of Product Characteristics. Bristol-Myers Squibb Pharmaceuticals Limited.

CSCC, Cutaneous squamous cell carcinoma; PD-1, Programmed cell death-1.

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MAT-GB-2004736(v1.0) Date of preparation: November 2020.

Primer on the Pathogenesis of Severe COVID-19: Part Two

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Disclosure: The author has declared no conflicts of interest.

Received: 23.06.20

Accepted: 27.08.20

Keywords: Angiotensin-converting enzyme 2 (ACE2), cluster of differentiation (CD) 147, coronavirus disease (COVID-19), myocarditis, NADPH oxidase, pneumonia, protein kinase R (PKR), severe acute respiratory syndrome (SARS), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).

Citation: EMJ. 2020;DOI/10.33590/emj/20-00159

Abstract

In the following continuation article, the author will expand on how the mechanisms discussed in Part One capitalise on host characteristics to produce the organ specific damage seen in severe coronavirus disease (COVID-19), with specific reference to pulmonary and cardiac manifestations. Pneumonia is the primary manifestation of COVID-19; presentation varies from a mild, self-limiting pneumonitis to a fulminant and progressive respiratory failure. Features of disease severity tend to directly correlate with patient age, with elderly populations faring poorest. Advancing age parallels an increasingly pro-oxidative pulmonary milieu, a consequence of increasing host expression of phospholipase A2 Group IID. Virally induced expression of NADPH oxidase intensifies this pro-oxidant environment. The virus avails of the host response by exploiting caveolin-1 to assist in disabling host defenses and adopting a glycolytic metabolic pathway to self-replicate.

Although not a cardiotropic virus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) can induce arrhythmias, a myocarditis-like syndrome, and myocardial infarction. Monocyte activation as a consequence of a surge of cytokine expression is the driver of these processes. Induced expression of cluster of differentiation 147 (CD147) and TNF-α may also have a role. SARS-CoV-2 fluently harnesses the immune mechanisms of the host to its advantage, rendering it a formidable systemic pathogen. Future effective treatments are contingent upon improved aetiological understanding.

INTRODUCTION

In Part One of this narrative review examining the pathogenesis of severe coronavirus disease (COVID-19), the author addressed the mechanism by which the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) virus subverts the innate immune response while remaining largely invulnerable to its effector functions. Critical SARS/SARS-CoV-2 infection is notable for an apparent biphasic (dysregulated) immune response, initially characterised by muted interferon-ß (IFNß) production which becomes robust and persistent (mostly derived from plasmacytoid dendritic cells) with the advent of clinical features. This response is associated with

impaired T-cell and antibody responses.[1] The virus itself is ostensibly invulnerable to cellular antiviral mechanisms, impeding all of them with the notable exception of the protein kinase R (PKR) pathway, which is activated in response to the intercellular presence of replicating double stranded (ds)RNA.[2-4] It is this PKR activation which amplifies IFNß expression and also causes copious overexpression of IL-6. This overexpression of IL-6 in a T-cell depleted milieu results in the characteristic cytokine storm (T-cell response would normally keep such cytokine storm in check).[5,6] The NLRP3 inflammasome pathway, as opposed to the mutually exclusive PKR, is activated in paediatric patients and leads to consequent milder manifestations.

The author also discussed the overproduction of cluster of differentiation 147 (CD147), also known as extracellular matrix metalloproteinase inducer or basigin, and how this dovetails with viral entry into host cells. The viral spike protein binds to the angiotensin-converting enzyme 2 (ACE2), precipitating the overproduction of NADPH oxidase as a downstream consequence. In Part Two, the focus is shifted to the systemic mechanisms of host-viral interaction.

THE GENESIS OF PULMONARY MANIFESTATIONS OF COVID-19

Much speculation regarding the noncardiogenic pulmonary oedema seen in COVID-19 centred on its physiological similarity to high altitude pulmonary oedema and its unconventional acute respiratory distress syndrome characteristics. This arose from a loose thread of comparison, prefaced on the presence of hypoxaemia that was out of proportion to the reported dyspnoea, the extent of the radiographic opacities, and a higher than typical respiratory system compliance on a ventilator (with reduced work of breathing). High altitude pulmonary oedema is characterised by exaggerated hypoxic pulmonary vasoconstriction and elevated pulmonary arterial pressures (45–65 mmHg). The latter is substantially at odds with the COVID-19 pneumonia phenotype, and these early speculations have been the subject of firm rebuke.[7-8]

The spectrum of COVID-19 pneumonia spans two phases, referred to as types L and H. The L-type perhaps best characterises the earlier stages of the infection when there is a loss of hypoxic pulmonary vasoconstriction while pulmonary arterial pressures remain near normal. The lungs remain very compliant in spite of worsening hypoxia, characterising the 'happy hypoxic' phenotype. During this phase, radiologically apparent subpleural and parafissural ground-glass opacification depict the limited extent of early oedema. However, it is the loss of hypoxic pulmonary vasoconstriction which accentuates the apparent ventilation/perfusion mismatch (vascular perfusion of the nonaerated lung). This eventuates in the H-type pneumonia, which is highly oedematous, has high elastance (low compliance), and a high right-to-left shunt phenotype.[9]

The Pro-Oxidant Pulmonary Milieu

The key feature that underpins the pathogenesis of lung disease in SARS/COVID-19 is a rather hostile pro-oxidant pulmonary micro-environment. The Perlman group[6] identified secreted phospholipase A2 Group IID (PLA2G2D) as a phospholipase that is preferentially and abundantly expressed in dendritic cells and lymphoid organs. This expression was enhanced in the lungs of aged animals. The source of this increase appeared to be largely CD11+ cells (i.e., respiratory dendritic cells, monocytes, and neutrophils). PLA2G2D is a 'resolving serum PLA2' that ameliorates dendritic cell-committed innate and adaptive immune responses by mobilising anti-inflammatory lipid mediators. In cases of SARS infection, when oxidative stress is enhanced, PLA2G2D is responsible for the pulmonary mobilisation of prostaglandin D2, which, by acting on its anti-inflammatory receptor D-type prostanoid receptor 1, dampens dendritic cell migration and thereby T-cell-driven antivirus response. In elderly populations wherein PLA2G2D levels are already high, viral clearance is impaired. Highlighting that SARS incurred this effect through oxidative stress, this group were able to demonstrate substantially ameliorated survival rates in aged (Bagg Albino [BALB]/c) mice exposed to the virus who were treated with the antioxidant N-acetylcysteine.[10] Mitochondrial reactive oxygen species, elaborated by host-viral metabolism and host antiviral response, are a known principal cause of hypoxic pulmonary vasoconstriction.[11] However, this enhanced hypoxic pulmonary vasoconstriction is contrary

to what is observed in COVID-19 pneumonia. Hence, a pro-oxidant milieu *per se* is insufficient to explain the phenotype.

The Role of IFN1 and Protein Kinase R

As established in Part One of this review, a late surge of IFN1 after peak viraemia provokes an escalation in monocyte-macrophage activation with concomitant curtailment of T-cell activation and antibody responses. The clinical consequence of this is worsened alveolar oedema and a failure to clear the virus efficiently.[12,13] It is also the case that papain-like proteases, along with other viral and induced host mechanisms, preclude production of IFN1.[2,14] This is certainly advantageous to the virus early in the course of infection when exposure to IFN1 might otherwise prevent viral replication. IFN production has been highlighted as biphasic. Critically, the IFN peak trails, rather than matches, peak viraemia.[12] The most likely explanation for this switch to elevated IFN1 expression is the emergence of PKR because of the presence of replicating dsRNA. Indeed, PKR is regulatory and may be required for IFN mRNA integrity.[15]

PKR promotes inducible nitric oxide synthase (iNOS) production via interferon regulatory factor-1 and NF-κB. iNOS reduces the hypoxic pulmonary vasoconstriction by relaxing pulmonary vascular smooth muscle.[16] Furthermore, the nucleocapsid protein of SARS-CoV activates the expression of cyclooxygenase-2 (COX-2).[17] iNOS specifically binds to COX-2 and S-nitrosylates it, enhancing COX-2 catalytic activity and thereby accentuating the inflammatory cascade.[18] This contributes to hypoxic pulmonary vasodilatation and the intense inflammatory process observed in COVID-19 pneumonia.

The Role of Caveolin-1

Caveolae are plasma membrane invaginations, which form in the Type 1 squamous alveolar cells lining the lungs and play a role in mechanoprotection. Caveolin-1 (Cav-1) is a scaffolding protein and a major component of caveolae. Molecular modelling and simulation of SARS-CoV has confirmed eight caveolin-binding sites.[19] Cav-1 has been touted, in at least one confirmatory study, as having the ability to induce protein-mediating viral endocytosis.[20] To date, no studies have been performed to evaluate the specific immune-pathogenic role of Cav-1 in SARS/SARS-CoV-2 infections. As such, it may be informative to extrapolate some data from the influenza A virus. The M2 matrix protein of human influenza A was shown to interact with Cav-1, facilitating Cav-1 influence on viral replication. Indeed, dominant-negative Cav-1 mutants resulted in a decrease in virus titre in infected cells.[21]

Enhanced Cav-1 expression may constitute a normal, adaptive response in host pulmonary epithelium. Cav-1 is a negative regulator of NADPH oxidase-derived reactive oxygen species.[22] As described in Part One of this review, angiotensin II binding to its Type 1 receptor, as a consequence of the viral spike protein binding to the ACE2 receptor, mediated enhanced signalling through various subtypes of NADPH oxidase to produce reactive oxygen species.[23,24] Also, Cav-1 was shown to suppress COX2 expression.[25]

Caveolin-1 and viral manipulation of host metabolism

Dominant-negative Cav-1-mutant mice have been shown to exhibit increased mitochondrial reactive oxygen species. However, 2-deoxy-D-glucose attenuated this increase, implicating that Cav-1 is in control of glycolytic pathways. Metabolomic analyses revealed that Cav-1 knockdown led to a decrease in glycolytic intermediates, accompanied by an increase in fatty acids, suggesting a metabolic switch.[26] Notably, a recent proteomic analysis of SARS-CoV-2-infected cells revealed host pathway changes such that a glycolytic profile was adopted. Glycolysis was necessary for viral replication, in that blocking glycolysis with nontoxic concentrations of 2-deoxy-D-glucose prevented SARS-CoV–2 replication in Caco–2 cells (a cancer cell line devoid of Cav-1).[27]

Caveolin-1 and mechanotransduction in the pulmonary epithelium

Cav-1 is a key regulator of pulmonary endothelial barrier function and is required for mechanical stretch-induced lung inflammation and endothelial hyperpermeability, both *in vitro* and *in vivo*. As such, Cav-1 has been shown to be central to the pathogenesis of pulmonary oedema in ventilator-induced lung injury.[28] Cav-1 is a major contributor to pulmonary

compliance.[29] The presence of hypoxia causes reduced Cav-1 expression as a routine adaptation of lung epithelial cells, leading to disassembly of cholesterol domains/caveolae.[30] The paradoxical overabundance of Cav-1 in the hypoxic pulmonary microenvironment of COVID-19 pneumonia may account for the clinical observation of high lung compliance in severely hypoxic patients, the so-called 'happy-hypoxics'.

Lung mechanical stretch employs a series of adaptive cellular elements. Components of the Hippo pathway, the transcription factors Yes-associated protein/Tafazzin (YAP/TAZ), were previously identified as key downstream elements and mediators of mechanical cues.[31] When a cell is subjected to mechanical stretch, large tumour suppressor kinase 1/2 (LATS1/2), which binds YAP/TAZ in the cytoplasm, prevents YAP/TAZ translocation to the nucleus where the transcription factor can positively influence cell division and other processes such as the induction of Cav-1 transcription.[32] In the setting of mechanical stretch, the co-chaperone protein BCL2-associated athanogene 3 (BAG3), facilitates the autophagic degradation of mechanically damaged cytoskeleton components. BAG3 utilises its WW domain to bind the YAP/TAZ inhibitors LATS1/2 or AmotL1/2 and thereby promotes nuclear translocation of YAP/TAZ, as well as concomitant transcriptional activation of proteins involved in cell adhesion and extracellular matrix remodelling, including Cav-1.[31,33] Cav-1, in turn, positively regulates YAP transcription.[34] Notably, YAP negatively regulates IFNβ expression and antagonises innate antiviral immunity.[35] BAG3 is a stress-inducible host protein that is specifically required for efficient replication of SARS-CoV.[36] The method through which BAG3 accomplishes this is unknown but may be similar to some herpes viruses. Varicella-zoster virus redistributes BAG3 and its co-chaperones Hsp70 and Hsp90 into nuclear replication/transcription foci in infected cells to efficiently complete its replicative cycle.[37]

Caveolin-1 and pulmonary hypertension

It should be noted that Cav-1 also functions as a negative regulator of pulmonary hypertension by inhibiting endothelial NOS (eNOS) uncoupling.[38] This may explain the near normal pulmonary arterial pressures seen in the context of apparently severe COVID-19 pneumonia. Murata et al.[39] reported that chronic hypoxia (10% oxygen levels for 1 week) induced the atrophy of endothelial cells, impaired calcium ion increase, and led to tight coupling between eNOS and Cav-1. This, in turn, blocked several eNOS-activation processes in the rat pulmonary arterial endothelium.[39] Furthermore, similar changes, such as atrophy of endothelial cells and condensation of eNOS into caveolae, were observed in hypoxic organ-cultured pulmonary endothelium.[40] This group demonstrated that dexamethasone could block this hypoxia-induced endothelial dysfunction in organ-cultured pulmonary arteries.[41] Dexamethasone disrupts glycolysis, likely through promotion of phosphofructokinase-1, which is likely to impede the host metabolism necessary for viral proliferation.[42] Dexamethasone may also exert possible beneficial effects through induction of claudin-4, which is protective of the alveolar epithelial barrier;[43] it may also suppress the virally-induced expression of NADPH-oxidase.[44] However, dexamethasone appears to promote the induction of Cav-1 in pulmonary epithelial cells, thereby exposing a limitation of its utility in COVID-19 pneumonia.[45]

As alluded to in Part One of this review, the production of haem oxygenase-1 also protects against oxidative lung injury. However, this protection is thwarted by Cav-1 expression through competitive inhibition.[46-48]

Although direct evidence for the role of Cav-1 has not been empirically demonstrated, there is a high likelihood that it plays a critical role in SARS-CoV-2 immunopathogenesis.

THE GENESIS OF CARDIAC MANIFESTATIONS OF COVID-19

For most patients, COVID-19 pneumonia constitutes the earliest and most virulent clinical manifestation of the condition. However, early in the course of the pandemic, it became apparent that cardio-specific manifestations such as myocarditis and arrhythmia constituted a major source of morbidity and mortality.[49,50] Although cardiovascular complications such as hypotension and tachycardia were common in patients with SARS, they were usually self-limiting. Bradycardia and cardiomegaly were less common, while cardiac arrhythmia was rare.[51,52]

During the Toronto, Canada, SARS outbreak in 2003, however, SARS-CoV viral RNA was detected in 35% of autopsied hearts.[53]

A clear departure from SARS infection has been witnessed with the high morbidity of cardiac manifestations of COVID-19. COVID-19, as a thromboinflammatory condition, may induce myocardial infarction.[54] Also, given the virulence of the pulmonary/systemic features of the condition, it is possible that those with an underlying cardiac condition might be induced to transition into cardiac failure.[55] These are rational assumptions; however, typically the cardiac manifestations of COVID-19 appear to trail behind the peak of the inflammatory processes when viral titres are in decline. This discussion will focus on the myocarditis-like syndrome because acute viral myocarditis can be fulminant and may sometimes mimic acute myocardial infarction and cardiac failure, as well as cause arrhythmias.[56]

Pathology of the COVID-19 Myocarditis-Like Syndrome

Myocarditis is an inflammatory disease of the myocardium and is diagnosed by established histological, immunological, and immunohistochemical criteria.[57,58] Histopathological reporting of endomyocardial biopsy and cardiac autopsy findings has been sparse and somewhat inconsistent during the pandemic so far.[59-61] The diagnosis has instead been prefaced on surrogate markers such as a raised troponin, electrocardiogram, and transthoracic echocardiogram changes.[62,63] However, no significant brisk lymphocytic inflammatory infiltrate, consistent with the typical pattern of viral myocarditis, has been apparent in any specimens examined so far.

Histopathological analysis of an endomyocardial biopsy specimen from a patient with COVID-19 myocarditis revealed sparse monocytic inflammatory infiltrates with significant interstitial oedema and limited focal necrosis.[60] One study even found endothelial cell infection in several organs, including the heart vessels, with no sign of lymphocytic myocarditis.[61]

Investigations have found that cardiac myocytes show nonspecific features consisting of focal myofibrillar lysis and lipid droplets. Viral particles in myocytes and endothelia were not observed, and small intramural vessels were free from vasculitis and thrombosis. Endomyocardial biopsies did not show significant myocyte hypertrophy or nuclear changes; interstitial fibrosis was minimal, focal, and mainly perivascular.[59] It should be noted that the sensitivity of endomyocardial biopsy for lymphocytic myocarditis is variable and depends on the duration of illness. In subjects with symptom duration of <4 weeks, up to 89% may have lymphocytic myocarditis,[64] but generally this is lower, between 10% and 35%, depending on the 'gold standard' used.[65-67]

There has been speculation that ACE2 expression is a likely reason for myocardial involvement in COVID-19.[68-70] This is questionable, given that no SARS-CoV-2 genome has been detected within myocardial cells, at least so far. Also, although the myocardium does express ACE2, its expression of TMPRSS2, which is necessary for viral entry, is negligible.[71-73] Further speculation around cardiac pericyte involvement inducing focal myocyte necrosis may be flawed, given that pericyte expression of TMPRSS2 is also modest. There is very limited evidence to suggest that SARS-CoV-2 is a cardiotropic virus.[68,74]

The Contribution of Monocytes

The specific role of monocytes in myocardial inflammation in COVID-19 infection may be caused by viral spike protein glycans binding to host monocyte lectins.[75,76] Alternatively, the macrophages seen in biopsy and autopsy specimens may be an enhanced population of cardiac resident macrophages. The development of advanced gene fate-mapping techniques has shown that, in the steady-state, two resident cardiac macrophage subsets are present: MHC-II^{low} CCR2- and MHC-II^{high} CCR2- cells. Under inflammatory conditions, a third macrophage subtype can be found in the heart and is classified as MHC-II^{high} CCR2+ cells. Originating completely from bone marrow-derived monocytes, the population of this macrophage subtype is recruited during inflammation and ultimately replaces embryo-derived cardiac macrophages because their proliferative properties diminish with age. Circulating CCR2+ monocytes interact with the CCR2 ligand, monocyte chemoattractant protein-1 (MCP-1/CCL2), which is a chemotactic cytokine that potentiates macrophage recruitment and invasion.[77,78] It should be noted that in addition to inflammatory (and reparative) processes, macrophages are key mediators of

electrical conduction in the heart and as such, their derangement by an inflammatory process may trigger arrhythmias.[79] MCP-1 is produced in excess as a consequence of the SARS-CoV-2-induced overproduction of PKR, both directly and through IL-6 overproduction, and by PKR-endoplasmic reticulum kinase which can promote MCP-1 production through activating transcription factor-4.[80-83] Bindarit is a safe inhibitor of MCP-1 and may be a useful to attenuate macrophage inflammatory activity in the context of cardiac disease seen in COVID-19.[84,85]

There is only sparse evidence for a substantive contribution to myocardial inflammation by infected T cells. It has been demonstrated that T cells may become infected by SARS-CoV-2, but virions are unable to propagate within T cells. The virus was demonstrated to enter T cells via the CD147 integral membrane receptor.[86]

The Contribution of CD147

In Part One of this review, the topic of IL-6 overproduction was addressed. One downstream consequence of this was the resultant induction of CD147.[87,88] CD147 has been shown to function as a signalling receptor for extracellular cyclophilins A and B and to mediate chemotactic activity of cyclophilins towards a variety of immune cells.[89] In this capacity, it has been demonstrated that in coxsackievirus B3 myocarditis, cyclophilin A/CD147 induces chemotaxis of T cells and monocytes/macrophages through matrix-metalloprotein-9 (MMP-9) induction. MMP-9 is required for adequate lymphocyte migration under inflammatory conditions and is thought to directly 'remodel' myocardium. When cyclophilin A is deleted, or in the presence of an antibody directed against CD147, there is reduced lymphocyte infiltration and myocardial infarct size, as well as preserved left ventricular function, in mice upon ischaemia and reperfusion injury.[90,91] Similar results have been extrapolated to humans with congestive heart failure, wherein remodelling secondary to MMP-9 plays a major role.[92] Thus, while it is not a specific viral effect, the induction of CD147 may be critical to the clinical manifestation of myocarditis.

Intriguingly, Cav-1 has been shown to negatively regulate CD147 clustering; however, this effect was most apparent at 4 °C and absent at body temperature.[93] It should be noted that azithromycin may be able to disrupt CD147 ligand interactions, establishing some of its basis in the treatment of malaria. However, given the inherent risks of QT prolongation in the context of an already diseased heart, caution would be advised with azithromycin in COVID-19. A preferable alternative might be meplazumab, an anti-CD147 humanised antibody, currently on orphan drug designation and approval by the U.S. Food and Drug Administration (FDA) for the treatment of malaria.[94]

The Putative Role of TNF-α

TNF-α, which may be overexpressed as a consequence of COVID-19-induced cytokine storm, aggravates myocarditis, and the neutralisation of TNF-α by antibodies or soluble receptors attenuates viral myocarditis.[95-98] Although the exact mechanism through which TNF-α contributes to decreased contractile performance in myocarditis is not well known, a number of studies have emphasised the negative impact mediated by NO.[99-101] It appears that IL-1α may co-operate with TNF-α to potentiate its effects in viral myocarditis.[97] In prolonged COVID-19 illness, the quantity of replicating virus starts to diminish and the relative burden of PKR expression is reduced. The protracted inflammatory process leads to some release of neutrophil serine proteases, such as neutrophil elastase, which processes pro-IL-1α to IL-1α independently of caspase (i.e., independently of inflammasome activation).[102-104] Furthermore, the diminished PKR signalling begins to surrender its inhibition of the NLRP3 pathway, which is triggered by the SARS viroporins ORF3a and ORF8b through cell membrane permeabilisation.[105,106]

The Role of the Renin-Angiotens in System

Because of viral spike proteins binding to ACE2 receptors, much has been made of the resultant effects on the renin-angiotensin system. There is a sustained boost in renin and angiotensin II expression in COVID-19.[107,108] These contribute to an endocrine backdrop that is supportive of the maintenance of the myocarditis-like condition. In this regard, angiotensin II receptor antagonists have been demonstrated to reduce myocardial damage in animal models of myocarditis.[109,110]

CANDIDATE PHARMACOTHERAPY

In establishing a platform for future treatments, real consideration needs to be given to antioxidation as a means of ameliorating the pro-oxidant milieu of the lungs in COVID-19 pneumonia. Lead candidates here would include N-acetylcysteine and the flavonoid quercetin. Quercetin has many desirable properties, including its lipid solubility in the surfactant rich environment of the lung, and it may have some efficacy in specifically blocking viral entry into cells and blocking airway epithelial cell chemokine expression, including MCP-1.[111-114] It may not be desirable to pharmacologically reduce Cav-1 expression because of the possible cardiac side effects.[115] The RECOVERY Trial, based in Oxford University, Oxford, UK, has found specific utility for dexamethasone as a significant treatment for severe COVID-19 pneumonia.[116] As discussed in Part One, elevated PKR was suggested to be amenable to remediation.[117] As relatively novel agents, bindarit and/or meplazumab may have a role in preventing/treating the myocardial injury seen in COVID-19.

CONCLUSION

In conclusion, the preceding narrative review has offered an overview of the pathogenesis of severe COVID-19 infection, as borne out through pulmonary and cardiac effects. The author acknowledges that all of the information synthesised in this review does need to be subjected to rigorous evaluation and investigation.

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Nutritional Management of Patients with Chronic Kidney Disease Through Low-Protein Diets

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Disclosure: Mr Jeffreys has received payment from EMJ to author the article. Miss Steinmair is a Medical Affairs Manager Renal Nutrition and is a paid employee of Dr Schär.

Support: The publication of this article was funded by Dr Schär.

Received: 28.10.20

Accepted: 24.11.20

Keywords: Dietary protein intake, gut microbiome, low protein, plant dominant.

Citation: EMJ. 2020;5[4]:66-72.

Abstract

Chronic kidney disease (CKD) is a global health problem, affecting approximately 10% of the adult population. It has a significant impact on patient quality of life and mortality rates, and increases costs for healthcare systems. Nutrition plays an important role in disease prevention, as it can help prevent hypertension and Type 2 diabetes mellitus, the two major underlying causes in CKD development. Medical nutrition therapy with protein reduction is an important pillar in the conservative management of patients with chronic renal failure and may improve overall patient outcomes. Its effects on uraemia, proteinuria, and metabolic acidosis have been demonstrated in numerous studies. Hence, the 2020 update to the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guideline for Nutrition in CKD has taken this into account, and recommends, with the highest level of evidence (1A), a diet with reduced protein intake in metabolically stable, nondialysis-dependent patients with CKD Stages 3–5. In practice, low-protein diets are often not particularly used for several reasons, such as concerns about the potentially increased risk for protein-energy wasting and poor adherence expectations. However, there is further evidence to show that a low-protein diet with adequate energy intake and high biological value protein supply, regularly followed by a trained dietitian, is safe and patient adherence increases with a personalised dietary approach, tailored to individual patient needs and considerations for dietary habits. Also, medical foods can help to facilitate reaching nutritional targets and preventing malnutrition, as they are a good source of almost nitrogen-free energy, providing only little amounts of protein, sodium, phosphorus, and potassium.

INTRODUCTION

Dependent on their level of residual kidney function and the presence of comorbidities, such as diabetes, hypertension,[1] and cardiovascular disease,[2,3] patients with chronic kidney disease (CKD) have differing nutritional requirements. A medical nutritional therapy, the so-called low-protein diet (LPD) maintains good nutritional status, reduces uraemic toxicity and metabolic alterations,[4] and decreases proteinuria,[5] and thus may play a part in lessening a declining kidney function and improving overall outcome. A plant-dominant, high-fibre, LPD may also bring additional benefits by altering the gut microbiome to enhance gut barrier performance, reduce inflammation, and delay CKD progression.[6]

LOW-PROTEIN DIET IN CHRONIC KIDNEY DISEASE

Recently, the National Kidney Foundation (NKF), in conjunction with the Academy of Nutrition and Dietetics (AND), published the 2020 update to the Kidney Disease Outcomes Quality Initiative (KDOQI) Clinical Practice Guideline for Nutrition in CKD.[7] A specific set of guidelines have been developed for the amount of protein recommended for CKD patient populations differentiated by diabetes:

- For those adults Stage 3–5, without diabetes, and who are metabolically stable and not on dialysis, patients should have an LPD of 0.55–0.60 g dietary protein/kg body weight/day, with or without ketoanalogues, or a very low-protein diet (VLPD) that provides 0.28–0.43 g dietary protein/kg body weight/day supplemented with ketoanalogues or amino acids.[7]
- Whereas for adult patients Stage 3–5, not on dialysis, and with diabetes, it is thought reasonable to prescribe a dietary protein intake of 0.60–0.80 g dietary protein/kg body weight/day to maintain a stable nutritional status and optimise glycaemic control.[7]

The most recent Kidney and Transplant Specialised Register Cochrane Review examined randomised controlled trials (RCT) for nondiabetic adults with CKD who had yet to require dialysis, and compared differing dietary protein regimes including VLPD (0.3–0.4 g/kg/day), LPD (0.5–0.6 g/kg/day), or normal protein intake (≥0.8 g/kg/day) for 12 months or more.[8] Evidence from 17 RCT (N=2,996) were examined, and it was concluded that VLPD compared with LPD, or normal protein diet, probably reduced the number of patients with advanced kidney failure who progress to dialysis.[8]

SUPPLEMENTING LOW-PROTEIN DIET WITH ESSENTIAL AMINO ACIDS AND KETOANALOGUES

LPD, especially if the protein amount is drastically restricted (VLPD), can be considered challenging to facilitate, owing to poor patient compliance and risk for protein-energy wasting (PEW) leading to malnutrition.[9] PEW is frequently encountered in patients with CKD and is distinguished by sarcopenia, weight loss, and low serum levels of albumin or transthyretin.[10]

In a Medline search for PEW in the title, Koppe et al.[10] identified 327 papers; a number of these specified PEW as a strong predictor of mortality in CKD. From this meta-analysis, the estimated prevalence of PEW in 16,434 haemodialysis patients ranged from 28 to 54%, and it increased as renal function declined.[10] To remove the risk of malnutrition and PEW in VLPD, ketoanalogues, precursors of essential amino acids, have been used as supplements. These analogues are converted into amino acids by transaminase while consuming ammonia molecules during this process. Aparicio et al.,[11] in their consensus statement for protein-restricted diets supplemented with keto acid therapy, advocated for the many benefits of following this strategy, including decreased uraemic toxins, reduced proteinuria, and improved insulin sensitivity. They have made recommendations for keto dosage depending on CKD staging.[11] A meta-analysis of 9 studies (N=410) with ketoanalogue supplementation was conducted by Jiang et al.,[12] who found there is a significant effect of supplemented VLPD/LPD in the protection of estimated glomerular filtration rate (eGFR), potentially reducing the progression of CKD.[12] Chewcharat et al.[13] conducted a more recent metanalysis of 17 RCT with over 1,400 patients and also concluded restricted protein diets supplemented with ketoanalogues helped preserve eGFR and

reduce proteinuria, serum phosphate, parathyroid hormone levels, blood pressure, and serum cholesterol. For patients to be able to benefit from such diet and lifestyle changes, centres have successfully deployed trained dietitians to counsel patients with CKD[14]

PHOSPHORUS HOMEOSTASIS AND AVOIDANCE OF SECONDARY PARATHYROIDISM

Patients with advanced CKD experience mineral disturbances as kidney function decreases. The ability to excrete phosphorus is reduced, leading to a positive phosphorus balance and triggering phosphaturia induced through increases in fibroblast growth factor 23 (FGF23) and parathyroid hormone.[15] Hyperphosphataemia is recognised as an independent risk factor for mortality in patients undergoing dialysis. A large observational study (n=3,490) was carried out in patients with CKD by Kestenbaum et al.[16] Associations between elevated serum phosphate, mortality risk, and myocardial infarction were found, which were independent of known confounding factors, including renal function.[16] After adjustment, they noted a relationship between serum phosphate levels >3.5 mg/dL and increased mortality risk; this increased linearly with each subsequent 0.5 mg/dL increase in serum phosphate levels.

To maintain serum phosphate levels in the normal range for patients with CKD Stages 3–5, it is recommended to restrict dietary phosphate to 800–1,000 mg/day.[7] However, the source of protein may have some bearing on the amount of available phosphorus. Moe et al.[15] reported on a trial in patients with CKD (n=8) to compare vegetarian and meat diets with equivalent nutrients prepared by clinical research staff. Results showed that after 1 week of a vegetarian diet, lower serum phosphorus levels and decreased FGF23 levels were achieved compared with a meat diet. It was concluded that protein source can have an impact on phosphorus levels in patients with CKD. Further, it was recommended that patients with CKD receiving dietary counselling should be informed about phosphate levels and protein sources for phosphates.[15] The phosphate in plant-based proteins is only 30–50% bioavailable due to being bound to phytates, compared with 70–80% bioavailability for animal-based foods, such as dairy.[16]

Sullivan et al.[17] reported on the risk of hyperphosphataemia due to consumption of phosphorus-rich food additives found in processed and fast foods; it is estimated that food additives contribute 30% of overall phosphate intake in a USA diet.[18] Sullivan conducted an RCT (N=279) in patients with end-stage renal disease that compared phosphorus levels; one group (n=145) were educated about avoiding phosphorus-rich food additives in groceries and fast foods, while the controls (n=134) followed usual care. They found that the educated patients resulted in a modest (0.6 mg/dL) but clinically significant improvement in serum phosphorus levels. Sullivan et al. concluded that this decline in average phosphorus level among the intervention cohort corresponds to a 5–15% reduction in relative mortality risk in observational studies.[17]

REDUCING METABOLIC ACIDOSIS IN CHRONIC KIDNEY DISEASE

Metabolic acidosis is a common complication in CKD and can trigger metabolic, endocrine, and musculoskeletal abnormalities.[19] Vegetable-based foods are rich in organic anion salts, which are directly absorbed by the gut to release bicarbonates. This results in a neutral or alkaline situation in the gut.[20] It has been estimated that consuming a fruit and vegetable diet can reduce acid excretion, equivalent to consuming 0.5 mEq/kg/day of sodium bicarbonate.[21] This can also lead to reduced proteinuria and decreased blood pressure in certain patients with CKD.[21] Extracellular sodium cations are responsible for fluid homeostasis, which is controlled through the renin–angiotensin–aldosterone system (RAAS).[7] This mechanism regulates sodium excretion through the kidneys and therefore exerts control on extracellular fluid volume and arterial blood pressure.[22] Excess sodium intake is excreted in the urine, with serum levels tightly controlled. However, in CKD, the system can be compromised by excessive dietary sodium consumption and/or kidney capability to excrete sodium becoming inadequate.

Humalda et al.[23] conducted a review to examine the evidence for the protective effect of dietary sodium restriction in patients with CKD specifically. In CKD cohorts, sodium intake is generally elevated, often above population average. For both diabetic and nondiabetic patients with CKD, a moderately lower sodium consumption was associated with better long-term outcomes of RAAS-blockade due to improved effects on proteinuria, independent of blood pressure. On the basis of an observed J-curve for sodium intake and outcome, with higher risk at both higher and lower sodium intakes, concerns have been expressed on the safety of rigorous sodium restriction. However, in their review, Humalda et al. concluded there are considerable potential benefits for most patients with CKD to have a moderately restricted sodium diet.[23] The KDOQI Clinical Practice Guideline for adults with CKD Stages 3–5 recommended that to control blood pressure and proteinuria, the amount of dietary sodium consumed should be <100 mmol/day (or <2.3 g/day).[7]

McMahon et al.[24] conducted a small, double-blind, placebo-controlled randomised crossover study of 25 nondialysed, nontransplanted patients with CKD. Using blood pressure, proteinuria, extracellular fluid volume, and arterial stiffness as markers of cardiovascular progression in CKD populations, they aimed to evaluate dietary sodium intake on these markers. They found a dietary sodium restriction of 60–80 mmol daily intake significantly decreased ambulatory blood pressure by 10/4 mmHg (systolic/diastolic) over the 24 hours. Also, for extracellular volume, albuminuria, and proteinuria, significant reductions were observed, with the latter two occurring independent of blood pressure changes. In this study, dietary sodium restriction reduced the incidence of most risk factors without significant adverse effects; however, symptomatic hypotension was observed and was resolved by modifying the antihypertensive regime.[24]

CARDIOVASCULAR RISK FACTOR MANAGEMENT IN CHRONIC KIDNEY DISEASE

In patients with CKD, inflammatory cytokine and advanced glycation end-product concentrations are often raised.[25] Susceptibility to inflammatory diseases, such as atherosclerosis and stroke, is heightened in this group of patients.[26] A vegetarian diet can confer cardiovascular benefits due to lower BMI, reduced blood pressure, a decreased incidence of hypertension, and reduced risk of Type 2 diabetes mellitus.[27] The large EPIC-Oxford study (n=37,875) grouped people into their expressed dietary preference for meat, fish, vegetarian, or vegan, and demonstrated age-adjusted mean BMI was highest in meat-eaters and lowest in vegans.[28] Several studies have reported reducing blood pressures when moving from a meat-based diet to a vegetarian one.[17,29]

Klahr et al.[30] conducted the Modification of Diet in Renal Disease (MDRD) Study in 840 patients to determine the effects of dietary protein restriction and blood pressure control on the progression of CKD. In Study 1, 585 patients with eGFR ranging from 25 to 55 mL/min/1.73m^2 were randomly assigned to usual protein diet or LPD and to usual or low blood pressure groups. After a mean of 2.2 years, the projected decline in eGFR did not differ significantly between diets or blood pressure groups. In Study 2, 255 patients with an eGFR of 13–34 mL/min/1.73m^2 were randomly assigned to LPD or VLPD and a usual or low blood pressure group. On follow-up, the VLPD group had a marginally slower decline in eGFR compared with the LPD group (p=0.07). In their conclusions, the authors stated there was no statistical difference between diet groups in time to end-stage renal disease or death.[30] A subsequent reanalysis by Levey et al.[31] suggested that the patient cohort on a diet of 1.30 g protein/kg/day, compared with the group allocated a diet of 0.58 g protein/kg/day and lower phosphorus intakes, experienced significantly more kidney function loss after the first 4 months from the start of the programme. After other known risk factors had been ruled out, a reduced protein consumption was associated with a 29% lower risk of CKD progression and no additional benefit from supplementation with ketoanalogues.[32] In the VLPD group, the causes of renal disease were hypertensive-vascular nephropathies (38%), glomerulonephritis (20%), tubule-interstitial nephropathies (15%), and unknown causes (27%); in the control group the percentages were similar: 41%, 18%, 16%, and 24%, respectively. Moreover, the frequency of cardiovascular complications (angina, infarction, stroke) was

46% in VLPD group, and 42% in control group (p=not significant).[5]

PLANT-BASED DIETS FOR PATIENTS WITH CHRONIC KIDNEY DISEASE

Kalantar-Zadeh et al.[33] have recently proposed a plant-dominant, low-protein diet as a pragmatic approach to facilitating CKD progression.[33] The proposition is for a diet that delivers daily protein ingestion of 0.6–0.8g/kg/day with at least 50% as plant-based sources, preferably whole unrefined and unprocessed foods; a low ~ sodium intake of <3 g/day; higher dietary fibre of 25–30g/day; and adequate nutritional energy intake of 30–35 kcal/kg/day.[33]

Potassium homeostasis and excretion are commonly impaired in patients with CKD, and hyperkalaemia is particularly concerning in late-stage CKD. Hyperkalaemia has an association with increased mortality and may contribute to peripheral neuropathy in patients with CKD.[7] The KDOQI recommendations for adults with CKD Stages 3–5 or post-transplantation should have dietary potassium intake adjusted to maintain normal serum potassium levels.[7] For adults with CKD Stages 3–5 or post-transplantation with either hyperkalaemia or hypokalaemia, dietary or supplemental potassium intake should be based on individual needs. It is suggested that when treating hyperkalaemia, the patient should recommend fruit and vegetables low in potassium.[7] It has been shown that double boiling can reduce the potassium concentration in potatoes and other tuberous root vegetables.[34,35] Eating a plant-dominant, high-fibre, low-protein diet may lead to favourable alterations in the gut microbiome. Among other things, the gut microbiota plays an essential role in the production of short-chain fatty acids. Dietary fibre enables the gut microbiota to generate short-chain fatty acids, which in turn become energy sources for gut bacteria and maintain intestinal epithelial barrier permeability.[36] Gut microbiome dysbiosis, resulting from alterations of composition and function of the gut microbiota and disruption of gut barrier function, is seen in pateints with CKD.[37] This gut flora disruption can generate large amounts of uraemic toxins, which can translocate into the systemic circulation due to the impairment of the intestinal barriers.[38] Uraemic toxaemia has been associated with the development of cardiovascular disease, CKD progression, and raised mortality risk in patients with CKD.[33,39]

PRACTICAL STRATEGIES

There is a need for practical applications in the management of dietary recommendations in CKD, which will turn the clinical guidance already set out into a set of pragmatic approaches. It is essential to engage with patients on their terms and make the clinical guidance a living reality for them.

Each diagnosis of CKD will require extensive and long-term changes to food and lifestyle for the patient. Virtually all dietary interventions require significant effort from the healthcare team, requiring expertise often outside the focus of most practising nephrologists.[40] Beto et al.[9] reported poor adherence to diet, medications, and treatments, estimated to vary between 20 and 70%, which could contribute to increased morbidity and mortality.[9] Delivering practical nutritional advice to patients with CKD requires co-ordination of many dietary components to cover calories, protein, carbohydrates, fats, electrolytes, and fluid, especially in patients with comorbidities, such as hypertension, diabetes, and cardiovascular diseases. Dietary intake studies have experienced adherence difficulties with the scope and complexity of CKD diet parameters. In Italy, nephrologists have a long-standing tradition of successfully implementing LPD for CKD. This has been due, in part, to the availability of low-protein foods, which have been available in Italy for decades. These products are carbohydrate-rich, low in salt, and virtually free of phosphorus, potassium, and nitrogen. They can effectively replace analogous food staples, such as bread, pasta, or biscuits, making it possible to reduce or replace protein of low biological value with higher value proteins, such as animal or legume proteins, and ensure a high energy intake.[41] D'Alessandro et al.[42] investigated the practicalities of such low-protein foods consumed by 100 patients with CKD prescribed LPD. Of these patients, 92% believed nutritional counselling with a trained dietitian was beneficial in successfully following their diet.[42] There are two main factors that enable successful LPD adherence: the prevention of PEW and the continual implementation of the dietary plan. For this reason, skilled teams

of nephrologists and dietitians are needed to frequently monitor patients on LPD and provide comprehensive nutritional support for their patients with CKD.[39]

Furthermore, D'Alessandro et al. mapped out simplified dietary regimens for these patients. They observed that low-protein products, which have a particularly favourable energy/phosphate ratio and can be high in plant fibre, are very important tools for the safe and successful implementation of dietary plans for patients with CKD.[14] Cost-effectiveness studies have been conducted to evaluate the economic benefits of pursuing a VLPD compared with dialysis in patients with Stage 5 CKD. Scalone et al.[43] followed 57 elderly patients with Stage 5 CKD who were randomised to dialysis or VLPD over 3 years. It was concluded that VLPD was a safe and beneficial strategy for these patients and allowed economic resources to be reallocated for further investments into the healthcare system.[43] Mennini et al.[44] compared the cost-effectiveness of LPD versus normal diet in patients with CKD Stages 4–5 over 10 years. A Quality-Adjusted Life Year (QALY) assessment showed LPD was both more effective in terms of good quality of life gained and was less expensive than normal diet.

CONCLUSION

Given the high incidence of CKD, nutritional therapy interventions have a significant role to play in delaying the progression of CKD, improving overall patient outcomes, and are significantly more cost-effective to healthcare systems compared with the cost of dialysis and transplantation. To deliver patient-centric dietary plans effectively, the involvement of dedicated dietitians in the patient management team is crucial. Food choice is a vital part of taking patients on this nutritional journey. Specific low-protein products, such as pasta, breads, and biscuits, can help patients to adhere to a LPD, assist in the facilitation of disease management, and provide better overall outcomes.

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Abbreviated Prescribing Information for Kyntheum® 210mg solution for injection in pre-filled syringe Please refer to the full Summary of Product Characteristics (SmPC) approved in your country before prescribing. ▼ This medicinal product is subject to additional monitoring. This will allow quick identification of new safety information. Healthcare professionals are asked to report any suspected adverse reactions. **Indication:** Treatment of moderate to severe plaque psoriasis in adult patients who are candidates for systemic therapy. **Active ingredient:** Each pre-filled syringe contains 210mg brodalumab in 1.5ml solution. 1ml solution contains 140mg brodalumab. **Dosage and administration:** *Posology: Adults:* The recommended dose is 210mg administered by subcutaneous injection at weeks 0, 1, and 2 followed by 210mg every 2 weeks. Consideration should be given to discontinuing treatment in patients who have shown no response after 12-16 weeks of treatment. Some patients with initial partial response may subsequently improve with continued treatment beyond 16 weeks. Each pre-filled syringe is for single use only. *Elderly:* No dose adjustment recommended. *Hepatic and renal impairment:* No dose recommendations can be made. *Children and adolescents below the age of 18 years:* Safety and efficacy of Kyntheum have not been established. *Method of administration:* Subcutaneous (SC) injection. Kyntheum should not be injected into areas where the skin is tender, bruised, red, hard, thick, scaly, or affected by psoriasis. The pre-filled syringe must not be shaken. After proper training in SC injection technique, patients may self-inject Kyntheum when deemed appropriate by a physician. Patients should be instructed to inject the full amount of Kyntheum according to the instructions provided in the package leaflet. **Contraindications:** Hypersensitivity to the active substance or to any of the excipients. Active Crohn's disease. Clinically important active infections (e.g. active tuberculosis). **Precautions and warnings:** *Inflammatory bowel disease (including Crohn's disease and ulcerative colitis):* Cases of new or exacerbations of inflammatory bowel disease have been reported with IL-17 inhibitors. Therefore, Kyntheum is not recommended in patients with inflammatory bowel disease. If a patient develops signs and symptoms of inflammatory bowel disease, or experiences an exacerbation of pre-existing inflammatory bowel disease, Kyntheum should be discontinued and appropriate medical management should be initiated. *Suicidal ideation and behaviour:* Suicidal ideation and behaviour, including completed suicide, have been reported in patients treated with Kyntheum. The majority of patients with suicidal behaviour had a history of depression and/or suicidal ideation or behaviour. A causal association between treatment with Kyntheum and increased risk of suicidal ideation and behaviour has not been established. Carefully weigh the risk and benefit of treatment with Kyntheum for patients with a history of depression and/or suicidal ideation or behaviour, or patients who develop such symptoms. Patients, caregivers and families should be advised of the need to be alert for the emergence or worsening of depression, suicidal ideation, anxiety, or other mood changes, and they should contact their healthcare provider if such events occur. If a patient suffers from new or worsening symptoms of depression and/or suicidal ideation or behaviour is identified, it is recommended to discontinue treatment with Kyntheum. *Hypersensitivity reactions:* Rare cases of anaphylactic reactions have been reported in the post-marketing setting. In the event of an anaphylactic reaction, or any other serious allergic reaction, administration of Kyntheum should be discontinued and appropriate therapy initiated. *Infections:* Kyntheum may increase the risk of infections. Caution should be exercised when considering the use of Kyntheum in patients with a chronic infection or a history of recurrent infection. Patients should be instructed to seek medical advice if signs or symptoms suggestive of an infection occur. If a patient develops a serious infection, they should be closely monitored and Kyntheum should not be administered until the infection resolves. Kyntheum should not be given to patients with active tuberculosis. Anti-tuberculosis therapy should be considered prior to initiation of Kyntheum in patients with latent tuberculosis. *Vaccinations:* It is recommended that patients be brought up-to-date with all immunisations in accordance with local immunisation guidelines prior to initiation of treatment with Kyntheum. Live vaccines should not be given concurrently with Kyntheum. The safety and efficacy of Kyntheum in combination with immunosuppressants, including biologics, or phototherapy have not been evaluated. **Drug interactions:** Live vaccines should not be given concurrently with Kyntheum. **Fertility, pregnancy and lactation:** *Women of childbearing potential:* Use an effective method of contraception during treatment and for at least 12 weeks after treatment. *Pregnancy:* There are no or limited amount of data from the use of brodalumab in pregnant women. As a precautionary measure, it is preferable to avoid the use of Kyntheum in pregnancy. Benefit risk for exposure of the infant to live vaccines following third trimester exposure to Kyntheum should be discussed with a physician. *Breast-feeding:* It is unknown whether brodalumab is excreted in human milk. A risk to the newborns/infants cannot be excluded. Whether to discontinue breast-feeding of discontinue Kyntheum therapy should be decided, taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. *Fertility:* No data are available on the effect of brodalumab on human fertility. **Adverse reactions:** *Common (≥1/100 to <1/10):* Influenza, tinea infections (including tinea pedis, tinea versicolor, tinea cruris), headache, oropharyngeal pain, diarrhoea, nausea, arthralgia, myalgia, fatigue, injection site reactions (including injection site erythema, pain, pruritus, bruising, haemorrhage). *Uncommon (≥1/1,000 to <1/100):* Candida infections (including oral, genital and oesophageal infections), neutropenia, conjunctivitis. *Rare (≥1/10,000 to <1/1,000):* anaphylactic reaction. **See SmPC for a full list of adverse reactions. Precautions for storage:** Store in a refrigerator (2°C-8°C). Do not freeze. Keep the pre-filled syringe in the outer carton in order to protect from light. Kyntheum may be stored at room temperature (up to 25°C) once, in the outer carton, for a maximum single period of 14 days. Once Kyntheum has been removed from the refrigerator and has reached room temperature (up to 25°C) it must either be used within 14 days or discarded. **Marketing authorisation number and holder:** EU/1/16/1155/001, LEO Pharma A/S, Ballerup, Denmark. **Last revised:** July 2020

Reporting of Suspected Adverse Reactions
Adverse reactions should be reported according to local guidelines.

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October 2020
MAT-38367

*PASI 100 at 12 weeks with Kyntheum®: 44% in AMAGINE-2 (N=612) and 37% in AMAGINE-3 (N=624). In the statistical analysis, missing data were imputed as nonresponses (NRI).[5]

LEO

Changing Paradigms in the Treatment of Advanced Urothelial Carcinoma: A 2020 Update

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Disclosure: Dr Linch is supported by the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre (BRC) and has received research funding from Bristol-Myers Squibb, AstraZeneca/MedImmune, Sanofi, and Astellas; and has received consulting fees from Janssen Pharmaceuticals and BioNTech. Dr Lee is supported by the National Institute for Health Research (NIHR). Dr Waterhouse has declared no conflicts of interest.

Received: 25.02.20

Accepted: 14.04.20

Keywords: Antibody-drug conjugates (ADC), bispecific antibodies, bladder cancer, enfortumab vedotin (EV), fibroblast growth factor receptor (FGFR), immunotherapy, nectin-4, targeted therapies, urothelial cancer.

Citation: EMJ. 2020:DOI/10.33590/emj/20-00044

Abstract

Advanced urothelial cancer (aUC) is invariably lethal and standard of care, platinum-based chemotherapy has changed little over the past 25 years. However, the past 5 years have been transformational with the advent of immunotherapies and targeted therapies. In this review, the authors focus on the therapies that are showing the greatest promise and have changed, or will imminently impact, the treatment landscape of aUC. Checkpoint inhibition is showing deep and durable responses in some patients and trial activity is concentrated on identifying the most suitable position within the treatment paradigm along with the most appropriate patients and therapeutic combinations. Novel targeted therapies in aUC are gaining renewed interest with nectin-4 antibody drug conjugates and fibroblast growth factor receptor inhibitors, both receiving recent regulatory approvals. Bispecific antibodies, capable of binding to two targets at the same time, are also showing promise. This review discusses the preclinical data, the relevant past, and present clinical trials along with regulatory status to provide a concise overview of the current and impending treatment options for aUC.

INTRODUCTION

Urothelial cancer is the 9th most common cancer in the world and the 10th most common cancer in the UK.[1] More than 10,000 new urothelial cancer cases occur in the UK every year, with a quarter of the patients presenting with locally advanced or metastatic urothelial carcinoma. Incidence rates are highest in older people (aged 85–89 years) and despite current treatment options the 5-year survival remains at only around 10%.[2]

The treatment landscape of advanced urothelial cancer ([aUC]: locally advanced or metastatic urothelial carcinoma) is now rapidly evolving with a recent increase in the number of approvals by the European Medicines Agency (EMA) and U.S. Food and Drug Administration (FDA) (Figure 1).

Timeline of FDA and EMA approvals of treatments for advanced urothelial carcinoma

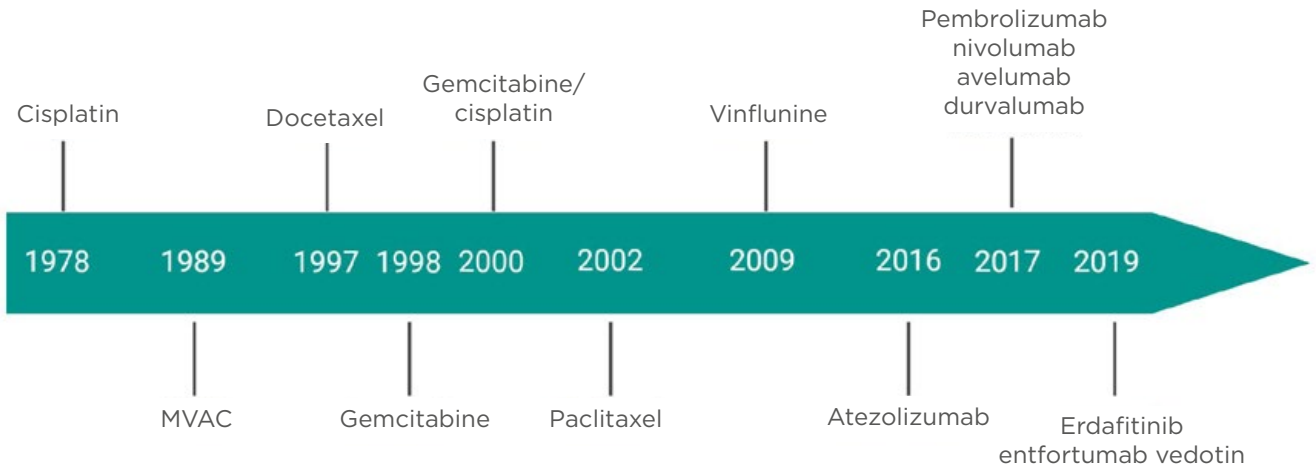

Figure 1: Timeline of approved agents in advanced urothelial cancer.

Agency (EMA) approvals of treatments for urothelial carcinoma.

EMA: European Medicines Agency; FDA: U.S. Food and Drug Administration.

This review summarises current treatment options for aUC, including cytotoxic chemotherapy and immune checkpoint blockade, with a focus on recent advances in targeted therapies and bispecific antibodies that are most likely to impact the management of aUC in the future.

CYTOTOXIC CHEMOTHERAPY

Platinum-based combination chemotherapy is currently the global first-line treatment for metastatic urothelial carcinoma.[3] Combinations in use include MVAC (methotrexate, vinblastine, doxorubicin, cisplatin), gemcitabine and cisplatin, and gemcitabine and carboplatin. Response rates have been reported at around 30–40%. Second-line chemotherapy agents such as taxanes, vinflunine, ifosfamide, and oxaliplatin have only demonstrated modest benefits.[4] For example, vinflunine, a microtubule inhibitor, led to only a 1.5 month improvement in progression-free survival (PFS) (median PFS: 3.0 versus 1.5; hazard ratio [HR]: 0.68; 95% confidence interval [CI]: 0.54–0.86; p=0.001) and 2.3 months improvement in overall survival (OS) (median OS: 6.9 versus 4.3 months; HR: 0.78; 95% CI: 0.61–0.99; p=0.04).[5] Immune checkpoint inhibitors (CPI) are now routinely used instead of cytotoxic chemotherapy in the second-line setting.

IMMUNOTHERAPY

The past 4 years has seen regulatory approval of five separate CPI (Figure 1) for the treatment of aUC. The authors have presented some of the stronger trial data to support second-line, first-line, and maintenance CPI; emerging data of combination checkpoint inhibition; and then focus on an interesting future advance, bispecific antibodies.

Immune Checkpoint Inhibitors

The strongest current evidence for the use of CPI in aUC comes from the KEYNOTE-045 study, which compared pembrolizumab (PD-1 inhibitor) with standard of care chemotherapy in patients who had previously progressed on platinum-based chemotherapy.[6,7] The co-primary endpoints were OS and PFS. With a median follow of 27.7 months, there was a 2.8 month improvement in survival with pembrolizumab compared to chemotherapy (median OS: 10.1 versus 7.3 months; HR: 0.7; 95% CI: 0.57–0.85; p<0.001) and in responders (response rate: 21.1% versus 11.0%) the median duration of response was substantially longer with the CPI (not reached versus 4.4 months). IMvigor211[8] was a Phase III study comparing atezolizumab (anti-PD-L1) to standard of care chemotherapy

in patients who had previously progressed on platinum-based chemotherapy. Atezolizumab was also active in the second-line setting but failed to reach the primary endpoint of improved OS in PD-L1 positive patients, partly because of the statistical design and better-than-expected performance of the chemotherapy control arm. Both agents are approved by the EMA for use in second-line treatment.

First-line CPI was initially tested in patients with aUC who were cisplatin-ineligible (renal impairment, neuropathy, or poor Eastern Cooperative Oncology Group [ECOG] performance status). This was following objective response rates (ORR) of 29% in the KEYNOTE-052 trial and 23% in the IMvigor 210 trials, two Phase II trials testing pembrolizumab and atezolizumab, respectively, in this setting,[9] which have led to the approval of these agents. The subsequent Phase III studies (KEYNOTE-361 and IMvigor130) compared chemotherapy with chemoimmunotherapy or immunotherapy alone in first-line metastatic disease. An interim analysis of these two studies suggested that CPI monotherapy may be less effective than chemotherapy in patients with low PD-L1 expression in the first-line setting,[10] leading to an EMA restriction of CPI monotherapy to patients with high PD-L1 expression. The initial results of IMvigor130, after a median of 11.8 months, have been reported in abstract form showing that atezolizumab plus chemotherapy leads to a 1.9-month improvement in PFS, the co-primary endpoint, compared to chemotherapy alone (median PFS: 8.2 versus 6.3 months; HR: 0.82; 95% CI: 0.70–0.96; p=0.007). There was a 2.6 month numerically higher OS, the other co-primary endpoint, for atezolizumab plus chemotherapy (median OS: 16.0 versus 13.4 months; HR 0.83; 95% CI: 0.69–1.00, p=0.027) but did not meet the prespecified interim boundary for significance. Outcomes of longer follow-up of IMVigor130 for OS remain unknown, but whether this is adopted will be contingent on the balance between survival and toxicity.[11]

Given the short PFS after first-line therapy and a significant fall off in the number of patients receiving second-line therapy, attributable to a decline in fitness, maintenance CPI has been tested in patients that had at least stable disease following 6–8 cycles of first-line platinum-based chemotherapy. Maintenance pembrolizumab led to a 2.6-month improvement in predicted median PFS (8.2 versus 5.6 months; p=0.023) compared to placebo.[12] Although not yet presented, Pfizer had announced following the planned interim analysis that the JAVELIN Bladder 100 study of avelumab maintenance versus standard of care met its co-primary endpoint prolonging OS in patients with PD-L1-positive tumours.[13] Given that there are now two positive studies in this setting, maintenance CPI may become a new standard of care.

Combination Immunotherapy

Given that response rates to CPI monotherapy are only in the order of 20–25%, other approaches are required to progress these agents. CPI is being tested in combination with chemotherapy as discussed above, but also with other CPI or with targeted therapies. However, these combination approaches have so far been disappointing, as exemplified by the DANUBE[14] and BISCAY[15] studies. A recent press release announced that the DANUBE study, a randomised Phase III trial of durvalumab (anti-PD-L1) and tremelimumab (anti-CTLA4) versus chemotherapy in first-line metastatic urothelial cancer failed to meet either of its co-primary endpoints, OS or OS in patients with high PD-L1 expression.[14] Two similar Phase III studies for first-line aUC are ongoing with the NILE study[16] testing triplet therapy (durvalumab and tremilimumab and chemotherapy), and the Checkmate901 testing an alternative doublet CPI (ipilimumab and nivolumab)[17] are still to report and help clarify whether CPI-CPI combinations or CPI-chemotherapy combinations are of benefit in aUC. In BISCAY,[15] a biomarker-driven Phase II study that explored either durvalumab monotherapy or durvalumab in combination with poly (adenosine diphosphate ribose) polymerase inhibitors, fibroblast growth factor receptor (FGFR)3 inhibitors, or mTOR inhibitors as second-line therapy in aUC, no combination treatment met the prespecified efficacy target.

Bispecific Antibodies

Bispecific antibodies are capable of binding to two targets at the same time.[18] These are early-stage molecules with most of the data derived from basic science and cell-line research, with some Phase I studies recruiting. One strategy of using a bispecific antibody is by targeting CD3 and a tumour antigen simultaneously. This can

recruit and activate T cells for effective tumour clearance by bringing the T cells closer to the cancer and activating them via the CD3 receptor pathway. Another strategy is to simultaneously target immune coinhibitory and costimulatory receptors. These receptors can be upregulated on activated T cells, including regulatory T cells, in the tumour microenvironment, and therefore using bispecific antibodies may increase the localisation of the antibodies to the tumour and improve tumour-specific clearance and reduce immune-related adverse events.

Preclinical data has suggested that bispecific antibodies may be a viable therapeutic option in urothelial cancer. B7-H3 is highly expressed on urothelial cancer cells. A CD3 and B7-H3 bispecific antibody armed on T cells demonstrated increased cytotoxicity towards bladder cancer cells. Secretion of IFN-γ and TNF-α was increased compared to unarmed activated T cells.[19,20] The same group demonstrated similar results with a bispecific antibody against CD3 and CD155 armed on activated T cells, again demonstrating their results on bladder cancer cell lines. MGD009 is a humanised anti-B7-H3 and anti-CD3 bispecific antibody that is being evaluated for safety in a multicentre, open-label, Phase I dose escalation and cohort expansion study[21] including patients with urothelial cancer. A Phase I study of MGD009 with an anti-PD1 antibody, MGA012[22] is currently recruiting.

A CTLA-4 and OX40 bispecific antibody resulted in T-cell activation and regulatory T-cell depletion *in vitro*.[23] Using syngeneic mouse models of bladder cancer, injections of this antibody resulted in durable tumour clearance.

LY3415244 is a TIM-3 and PD-L1 bispecific antibody. It is hoped that intrinsic and acquired resistance to immune checkpoint inhibition can be overcome by targeting and inhibiting both these co-inhibitory receptors. J1C-MC-JZDA is a multicentre, nonrandomised, open-label, Phase Ia/Ib study assessing LY3415244. The Phase Ia study will recruit patients with any tumour type and the Phase Ib expansion cohorts will recruit patients who have previously received a PD-1 or PD-L1 inhibitor, in non-small cell lung cancer, urothelial cancer, and melanoma.[24] Similarly, RO7121661 is an anti-PD-1/TIM-3 bispecific antibody that entered Phase I studies for treatment of patients with metastatic solid tumours.[25] Bispecific antibodies targeting other immune co-inhibitory checkpoints are running in Phase I studies. MGD013 targets PD-1 and LAG-3 and is recruiting to Phase I.[26]

TARGETED THERAPIES

Multiple targeted agents, including small molecule inhibitors or antibodies, have been tested against vascular endothelial growth factors, epidermal growth factor receptors, mTOR, and the cell cycle. Unfortunately, none of these agents demonstrated sufficient activity or efficacy in trials to gain regulatory approval (Table 1).[6,27-43] Recently, two targets, nectin-4 and FGFR, have demonstrated great promise and are discussed in more detail here.

Nectin-4

Antibody–drug conjugates (ADC) enable the delivery of high concentrations of cytotoxic chemotherapy to tumour cells by enabling targeted delivery through conjugation with a monoclonal activity. This strategy has become a standard of care in some malignancies, for example TDM-1 in breast cancer.

Enfortumab vedotin (EV) is an ADC that binds to nectin-4, a transmembrane protein that regulates a number of cellular functions including angiogenesis, and is highly expressed in multiple tumours including urothelial, ovarian, lung, breast, and gastric.[44-46] Upon binding to nectin-4, EV is internalised into the cell where cytoplasmic proteases cleave the linker between the nectin-4 antibody and the drug payload monomethyl auristatin E (MMAE) (vedotin is an MMAE and a protease-cleavable linker to an antibody),[47] releasing its cytotoxic activity. More specifically, MMAE inhibits tubulin polymerisation, leading to mitotic arrest and downstream apoptotic cell death.

Given that nectin-4 is expressed by 97% of aUC,[48,49] a global, Phase II, single-arm study of EV in patients with aUC who had previously been treated with platinum-based chemotherapy and anti-PD-1 or PD-L1 immune CPI was performed.[48] Here, 125 patients were treated with EV that was administered intravenously on Days 1, 8, and 15 of a 4-week cycle.

Table 1: Summary of clinical trials of targeted agents in advanced urothelial cancer that have not gained regulatory approval.[6,27-43]

Author Study name	Phase	Targeted agent	Primary endpoint	Patient selection	Treatment	Patients (n)	Response and survival
Petrylak et al., 2020[27] RANGE"	III	Ramucirumab (anti-VEGFR2 antibody)	PFS	mUC, refractory to platinum-based chemotherapy	Docetaxel and ramucirumab versus docetaxel and placebo	530	Ramucirumab/ docetaxel versus placebo/docetaxel: RR: 24.5% versus 14.0% PFS: 4.07 months versus 2.76 months Median OS: 9.4 months versus 7.85 months
Rosenberg et al., 2020[28] CALGB 90601 (Alliance)	III	Bevacizumab (anti-VEGF antibody)	OS	mUC, first line	Gemcitabine and cisplatin with bevacizumab or placebo	506	PFS HR: 0.77 (95% CI: 0.63–0.93) OS: 14.5 (GCB) versus 14
Grivas et al., 2014[29]	II	Sunitinib (multiple kinase inhibitor including PDGF-R and VEGFR)	% with progression at 6 months	Advanced UC post primary chemotherapy	Maintenance sunitinib versus placebo	54	Sunitinib versus placebo: 6 months progression rate: 71.7% versus 64.3%. Median PFS: 2.9 months versus 2.7 months Median OS: 10.5 months versus 10.3 months
Bellmunt J et al., 2011[39]	II	Sunitinib	TTP safety	First-line in UC, ineligible to cisplatin	Sunitinib	41	PR: 8%; SD: 50% (45% of them ≥3 months) Median TTP: 4.8 months Median OS: 8.1 months
Gallagher et al., 2010[31]	II	Sunitinib	ORR	mUC, post chemotherapy	Sunitinib 37.5 mg continuously (Cohort B) versus 50 mg for 4 weeks with 2 weeks off (Cohort A)	78	PR in 3/45 patients in cohort A and 1/32 patients in Cohort B. Clinical regression or stable disease: 43%. PFS: 2.4 months versus 2.3 months OS: 7.1 months versus 6.0 months

Table 1 continued.

Author Study name	Phase	Targeted agent	Primary endpoint	Patient selection	Treatment	Patients (n)	Response and survival
Bellmunt et al., 2017[6] Wong et al., 2012[32]	II	Cetuximab (anti-EGFR antibody)	PFS	mUC, pretreated with one line of chemotherapy	Cetuximab with or without paclitaxel	41	PFS monotherapy versus combination: 7.6 versus 16.4 OS: 17 versus 42 ORR: 25 in combination
Hussain et al., 2014[33]	II	Cetuximab	ORR	Advanced UC	Gemcitabine/ cisplatin with or without cetuximab	88	Gemcitabine/ cisplatin versus combination with cetuximab: ORR: 57.1 versus 61.4 Grade 3–5 AE: 75 versus 83 Median PFS: 8.5 versus 7.6 Median OS: 17.4 versus 14.3 Monotherapy arm was closed
Miller et al., 2016[34]	II	Gefitinib (TKI against EGFR)	TTP	Advanced UC, in combination with first-line chemotherapy	Gemcitabine and cisplatin chemotherapy with concomitant gefitinib (Arm A), sequential gefitinib (Arm B), or alone (Arm C)	105	Median TTP for arms A, B, and C were 6.1, 6.3, and 7.8 months, respectively
Choudhury et al., 2016[35]	II	Afatinib (TKI against HER2/EGFR)	PFS	mUC, platinum-refractory	Afatinib 40 mg/day continuously until progression or intolerance	437	Publication of initial results of 23 patients: 21.7% met PFS3 (2/23 PR; 3/23 SD) 83.3% with *HER2* and/or *ERBB3* alterations achieved PFS3 versus 0/15 patients without alterations ($p<0.001$) Median TTP/ discontinuation was 6.6 months in patients with *HER2/ ERBB3* alterations versus 1.4 months in patients without

Table 1 continued.

Author Study name	Phase	Targeted agent	Primary endpoint	Patient selection	Treatment	Patients (n)	Response and survival
Bellmunt et al., 2015[36] Powles et al., 2017[37]	II/III	Lapatinib (TKI against HER2/EGFR)	PFS	mUC after first line chemotherapy, HER 1/2-positive	Maintenance lapatinib versus placebo	232	Median PFS for lapatinib and placebo was 4.5 versus 5.1 months OS for lapatinib and placebo was 12.6 months and 12.0 months
Hussain et al., 2007[38]	II	Trastuzumab (anti-HER2 antibody)	Toxicity	Advanced HER2/neu-positive UC	Trastuzumab in combination with paclitaxel, carboplatin, gemcitabine	40	Provisional results from publication: Most common Grade 3 or 4 was myelosuppression Grade 3 sensory neuropathy occurred in 14.0%. Grade 1–3 cardiac toxicity was 22.7% Therapy-related deaths (n=3) ORR: 70% (CR [n=5], PR [n= 26], confirmed responses [n=25]) Median TTP and survival were 9.3 months and 14.1 months
Hainsworth et al., 2018[39] Bryce et al., 2017[40] MyPathway	IIa	Trastuzumab/pertuzumab (anti-HER2/HER3 dimerisation antibody)	OS % of Atezolizumab-treated patients with tTMB ≥16 mutations/Mb with OR	HER2-positive mUC	Trastuzumab/pertuzumab, erlotinib, vemurafenib/cobimetinib, vismodegib, alectinib, and atezolizumab	765	Recruitment ongoing. Preliminary results: At median FU 5.4: 1 patient had CR, ongoing at 12.5 months; 2 PR; DOR 3.7 and 5.5 months, 2 SD for >4 months
Rose et al., 2018[41]	II	Palbociclib (CDK4/6 inhibitor)	PFS	mUC after failure of first-line chemotherapy	Palbociclib	12	Two patients (17%) achieved PFS4 with insufficient activity to advance to Stage 2 No responses were seen Median PFS: 1.9 months Median OS: 6.3 months

Table 1 continued.

Author Study name	Phase	Targeted agent	Primary endpoint	Patient selection	Treatment	Patients (n)	Response and survival
Milowsky et al., 201342	II	Everolimus (mTOR inhibitor)	PFS-2 safety and toxicity	Metastatic TCC	Everolimus	46	Most common Grade 3/4 toxicities were fatigue, infection, anaemia, lymphopenia, hyperglycaemia and hypophosphataemia PR in nodal metastases (n=2), with 1 achieving a 94% decrease in target lesions and remaining on drug at 26 months Minor tumour regression (n=12)
Niegisch et al., 201543 AUO Trial AB 35/09	II	Everolimus	RR	Second-Line treatment of advanced UC	Paclitaxel and everolimus	28	ORR: 13% PFS: 2.9 months Median OS: 5.6 months

AE: adverse event; CR: complete response; CPS: combined positive score; DOR: duration of response; EGFR: epidermal growth factor receptor; FGFR: fibroblast growth factor receptor; FU: follow-up; GCB: germinal centre B-cell; HR: hazard ratio; HER2: human epidermal growth factor receptor; mTOR: mammalian target of rapamycin; mUC: metastatic UC; OR: overall response; ORR: overall response rate; OS: overall survival; PDGF-R: platelet-derived growth factor receptor; PD-L: programmed death ligand; PFS: progression-free survival; PR: partial response; RR: response rate; SD: stable disease; TEAE: treatment-emergent adverse events; TKI: tyrosine kinase inhibitor; tTMB: tissue tumour mutational burden; TTP: time to progression; UC: urothelial cancer; VEGFR: vascular endothelial growth factor receptor; 95% CI: 95% confidence interval.

The primary endpoint was ORR using the Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1 criteria. Secondary endpoints included duration of response, PFS, OS, safety, and tolerability.

Nectin-4 expression was assessed by immunohistochemistry (using a modified H-score, a continuous weighted scale).[50] The ORR was 42% (95% CI: 35.1–53.2%), with 12% complete responses, which is markedly higher than any other third-line treatment that has been tested in aUC. The median duration of response was 7.6 months (range: 0.95–11.30 months). The estimated median PFS was 5.8 months (95% CI: 4.9–7.5 months) and the estimated median OS was 11.7 months (95% CI: 9.1 months to not reached). As well as this prolonged survival compared to historical controls, EV was also well tolerated with the most common grade ≥3 toxicities being neutropenia (8%), anaemia (7%), and fatigue (6%). Treatment-related adverse events led to dose reductions in 32% of patients and discontinuation of treatment in only 12% of patients, with no treatment-related deaths. The most common toxicities of any grade were fatigue (50%), alopecia (49%), decreased appetite (44%), dysgeusia (40%), peripheral sensory neuropathy (40%), nausea (40%), diarrhoea (40%), and maculopapular rash (27%). Based on results from the EV-201 trial, the FDA granted accelerated approval to EV in December 2019. The global registration Phase III study of third-line EV compared to standard of care chemotherapies (EV-301) has recently completed data accrual

and initial results are expected towards the end of 2020.[51]

Given this encouraging level of activity in the third-line setting, EV has also been tested in combination with pembrolizumab (anti-PD-1) as a first-line therapy in patients who were ineligible to receive platinum chemotherapy. Initial results were presented at ESMO 2019,[52] and updated at GU ASCO 2020.[53] The overall response rate was 73.3% with a complete response rate of 15.6%. At a median follow up of 10.4 months, 55% of the responders had an ongoing durable response. Treatment-related toxicities included fatigue (58%; 11% ≥G3), alopecia (53%), and peripheral sensory neuropathy (53%; 4% ≥G3). However, one patient in the study died as a result of multiple organ failure. The FDA granted breakthrough designation to the EV and pembrolizumab combination in February 2020. This combination demonstrates encouraging efficacy compared to previous drugs used in the first-line setting and, if replicated in Phase III studies, could lead to a paradigm shift in the treatment of aUC.[52,53] The EV-302 Phase III study will evaluate the EV and pembrolizumab combination therapy versus standard of care gemcitabine and platinum in the first-line treatment setting for aUC, with primary outcome measures of PFS and OS.

Fibroblast Growth Factor Receptor

FGFR activation results in signal transduction via the downstream MAPK and PI3K pathways, which regulate tumour survival and growth.[52] FGFR3 alterations are present in approximately one-fifth of patients with urothelial bladder cancer and in one-third of patients with upper tract urothelial carcinomas.[54]

Erdafitinib is a potent inhibitor of FGFR1–4 and a weaker inhibitor of VEGFR2. It has been the first targeted anticancer therapy to gain accelerated approval by the FDA[55] for patients with aUC carcinoma with susceptible *FGFR2* or *FGFR3* mutations, based on the results of the BLC2001 study.[56] Simultaneously, approval for the Therascreen® (Qiagen, Hilden, Germany) FGFR RGQ RT-PCR Kit was given as the companion diagnostic. This is a reverse-transcriptase-PCR assay that tests for specific *FGFR3* mutations or *FGFR2/3* fusions using RNA extracted from formalin-fixed paraffin-embedded samples.

The BLC2001 study was an open-label Phase II study enrolling patients with aUC with prespecified *FGFR* alterations[56] to treatment with oral erdafitinib. In total, 99 patients with specified *FGFR3* gene mutations or *FGFR2/3* gene fusions were recruited. Patients had to have progressed following treatment with one course of chemotherapy or within 12 months after neoadjuvant or adjuvant chemotherapy. The primary endpoint was ORR and secondary endpoints included duration of response, PFS, and OS.

Patients were initially randomised in a 1:1 ratio to either receive an intermittent regimen (10 mg per day, with daily administration for 7 days and off for 7 days) or a continuous regimen (6 mg per day). Subsequently, a planned interim analysis of safety and efficacy was performed in June and July 2016 and further enrolment to the intermittent-regimen group was halted. In August 2016, the study was converted to a single-group analysis following a protocol amendment to increase the starting dose to 8 mg per day in a continuous regimen.

The ORR was 40% (95% CI: 31–50), the median duration of response was 5.6 months (95% CI: 4.2–7.2). The median PFS was 5.5 months (95% CI: 4.2–6.0) and median OS was 13.8 months (95% CI: 9.8–not reached). Patients with *FGFR3* mutations were noted to have a better ORR (49%) compared to those with *FGFR2/3* fusions (16%).

In terms of safety, 46% of patients experienced a treatment-related adverse event at Grade 3 or higher. The most commonly reported toxicities that were Grade 3 or higher were hyponatraemia (11%), stomatitis (10%), and fatigue (7%) and 13 patients had treatment discontinuation. This was because of detachment of the retinal pigment epithelium, hand-foot syndrome, dry mouth, and skin or nail events. Furthermore, 55 patients required a dose reduction, which was commonly a result of stomatitis (16 patients) and hyperphosphataemia (9 patients). Common adverse events included hyperphosphataemia (77% all grade), stomatitis (58% all grade), diarrhoea (51% all grade), and dry mouth (56% all grade). Hand-foot syndrome was at 23% any grade. Hyperphosphataemia, a class effect of FGFR inhibition,[57] which is thought to be secondary to inhibition of FGF23 signalling,[58]

could be a useful pharmacodynamic biomarker. A randomised Phase III study (THOR)[59] is now investigating the benefit of erdafitinib compared with chemotherapy or pembrolizumab, with a primary outcome of OS. Patients who have progressed on or after one or two prior treatments, at least one of which includes an anti-PD-1/PD-L1 agent (Cohort 1) or one prior treatment not containing an anti-PD-1/PD-L1 agent (Cohort 2).

Other FGFR inhibitors include infigratinib, rogaratinib, pemigatinib, and Debio 1347 (Debiopharm, Lausanne, Switzerland). Infigratinib (BGJ398) is a potent and selective FGFR1–3 inhibitor.[60] An exploratory analysis of Phase II data[61] demonstrated a difference in ORR in upper urinary tract urothelial carcinoma (50%) compared to lower urinary tract urothelial carcinoma (22%). The difference in ORR could be because of differences in genomic alterations between the two patient groups. A higher frequency of *FGFR3-TACC3* fusions (12.5% versus 5.8%) and *FGFR3 R248C* mutations (50% versus 11.5%), and a lower frequency of *FGFR3 S249C* mutations (25% versus 59.6%) was found when comparing upper with lower urinary tract urothelial carcinoma.

Rogaratinib (BAY1163877) is a potent and selective inhibitor of FGFR 1–4.[62] Results from the Phase I study were reported in 2016.[63] A Phase III trial[64] comparing rogaratinib against chemotherapy in metastatic urothelial carcinoma who have received prior platinum-based chemotherapy is currently active but not recruiting in January 2020.

The interim results of the FIGHT-201[65] study, a Phase II, open-label, multicentre study of pemigatinib (INCB054828), was reported in 2018. Patients had to have previously progressed on one or more treatments and had *FGFR3* mutations or fusions (Cohort A) or other *FGF/FGFR* gene alterations (Cohort B). 64 patients were in Cohort A and with an ORR of 25% (95% CI: 14–40%). There were no responses determined by RECIST 1.1 in Cohort B. FIGHT-205,[66] looking at pemigatinib plus pembrolizumab versus pemigatinib alone versus standard of care for participants with metastatic or unresectable urothelial carcinoma who are not eligible to receive cisplatin, are harbouring *FGFR3* mutation or rearrangement, and who have not received prior treatment, is currently recruiting.

Debio 1347 is a selective inhibitor of FGFR 1–3. The FUZE trial is an ongoing Phase II basket trial in *FGFR* fusion-positive advanced solid tumours irrespective of tumour histology, enrolling patients with aUC with at least one prior treatment line.[67] The primary endpoint is ORR.

Targeting FGFR3 alone in pretreated patients has not demonstrated similar levels of ORR compared to the multitargeted FGFR inhibitors. A Phase II study of dovitinib, a multitargeted tyrosine kinase inhibitor with activity against FGFR3 looked at 44 aUC patients who progressed after at least one chemotherapy regimen.[68] Patients were classified as *FGFR3* mutant or wild type. The study was not taken further because of a lack of ORR (0%; 95% CI: 0.0–26.5).

Small molecule tyrosine kinase inhibitors are not the only strategy to target the FGFR pathway in urothelial carcinoma. Vofatamab (B-701) is a fully human monoclonal antibody against FGFR3 that blocks activation of the wild type and genetically activated receptor. FIERCE-21 is a Phase Ib/2 study designed to evaluate vofatamab monotherapy or in combination with docetaxel[69] in metastatic urothelial carcinoma with at least one treatment failure. The follow-up is immature at this time; however, data presented at ASCO GU 2019 showed that five out of 21 patients have had a partial response in the vofatamab combination arm compared to one out of 21 patients in the monotherapy arm.

CONCLUSION

This review discussed strategies that allow better targeting of aUC. ADC in the form of EV demonstrate good response rates in pretreated metastatic disease, and early results in the first-line setting are encouraging. There are now actionable genomic alterations in the form of FGFR inhibitors that can lead to better outcomes in selected groups of patients. Bispecific antibodies may allow urothelial cancer cells to be targeted specifically and overcome mechanisms of resistance to immune CPI.

There have been advances in developing targeted and personalised therapies in metastatic urothelial carcinoma. Further discussed here were

three different targeted agents demonstrating promise in both clinical trials and preclinical research (Figure 2).

The development of targeted agents in aUC has positive implications for patients' outcomes and treatment options. The challenge remains in optimising patient selection, sequencing of treatment, and whether combination strategies can lead to better outcomes. This represents a paradigm shift in the treatment of metastatic urothelial cancer, where previously treatment options were limited to cytotoxic chemotherapy.

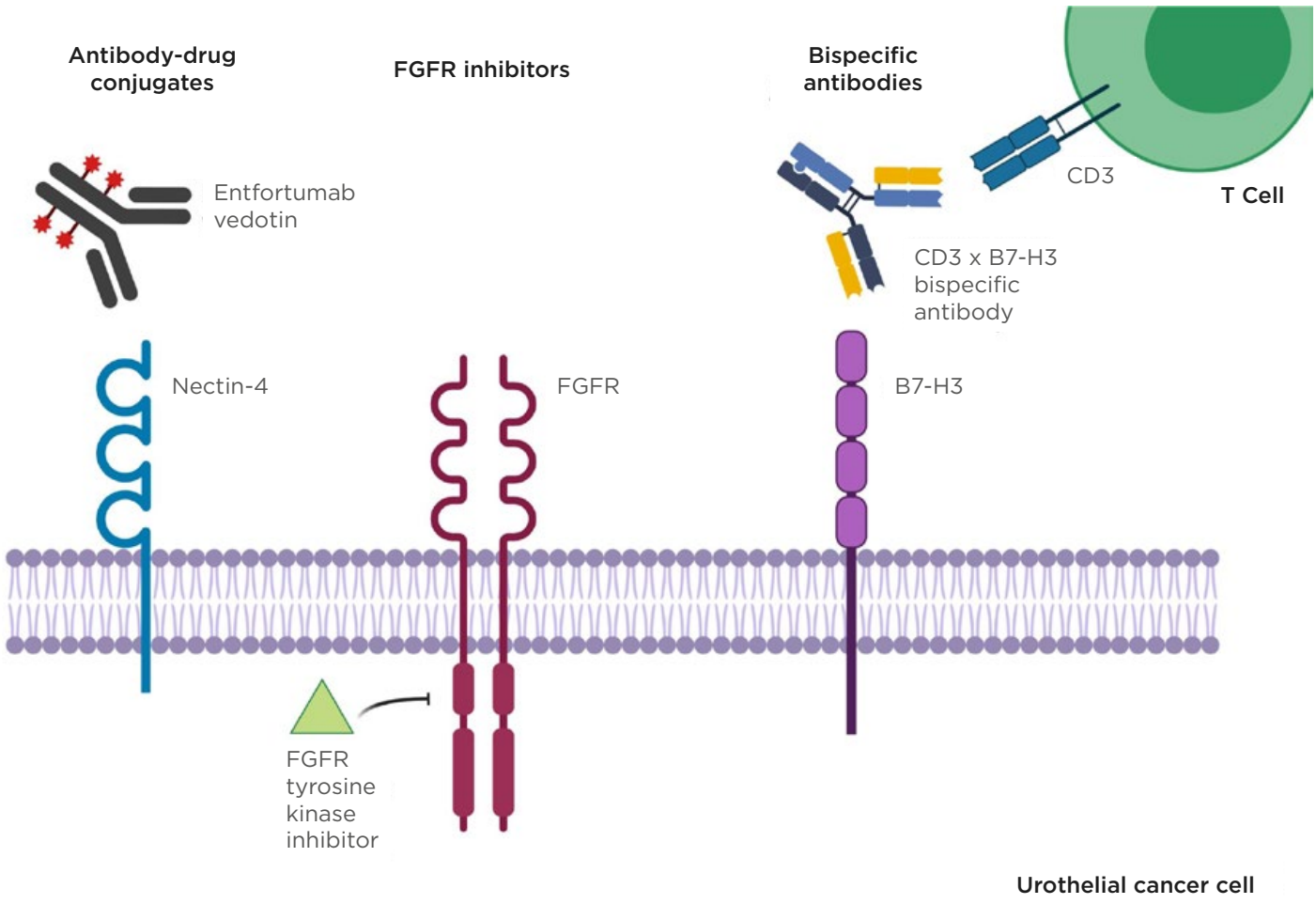

Figure 2: Promising targeted treatment strategies in advanced urothelial cancer.
FGFR: fibroblast growth factor receptor.

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Optimising Response to Advanced Therapies in Rheumatoid Arthritis – Using Prehabilitation to Improve Success?

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Disclosure: Prof Edwards has received honoraria, attended advisory boards, been a member of a speakers bureau, and received research support from AbbVie, BMS, Biogen, Celgene, Fresenius, Gilead, Janssen, Lilly, Mundipharma, Pfizer, MSD, Novartis, Roche, Samsung, Sanofi, and UCB. The other authors have declared no conflicts of interest.

Received: 24.04.20

Accepted: 24.07.20

Keywords: Advanced therapies, biologics, BMI, exercise, JAK inhibitors (JAKi), obesity, prehabilitation, psychological support, smoking.

Citation: EMJ. 2020;5[4]:87-95.

Abstract

In recent years, a new concept of prehabilitation, enhancing an individual's functional capacity ahead of a medical intervention, has begun to be explored in the fields of surgery and oncology, with positive results. This article explores applying the principle of prehabilitation to patients with rheumatoid arthritis prior to starting advanced therapies, including biologic disease-modifying antirheumatic drugs and targeted synthetic disease-modifying antirheumatic drugs. In this article, the literature is reviewed and the existing evidence is summarised, and the suggestion is that this approach could improve a patient's chance of achieving low disease activity or remission.

There are a number of opportunities for improving the likelihood of patients with rheumatoid arthritis having a good response to therapy. Research shows that smokers starting TNF inhibitors are less likely to achieve a good response compared to non-smokers. Obese patients are also less likely to achieve a good response with TNF inhibitors; female patients with obesity may be less likely to achieve a good response with tocilizumab and early real-world data suggest there may be a reduced response to JAK inhibitors. Rheumatoid arthritis patients experiencing depression are less likely to respond to TNF inhibitors. Increased physical activity is potentially beneficial for all rheumatoid arthritis patients, although the effect on response to specific drugs has been less widely explored.

Prehabilitation approaches could include targeting smoking cessation, improving physical activity, providing psychological support, optimising BMI, and dietary changes. A number of studies have shown that each of these interventions can lead to significant improvements in disease activity scores, with some patients potentially benefitting from more than one intervention. The authors identify principles for delivering prehabilitation in practice and suggest that this is an exciting area for ongoing research.

INTRODUCTION

In recent years, there has been a growing body of evidence that prehabilitation, enhancing an individual's functional capacity ahead of a medical intervention; encompassing medical optimisation, physical exercise, nutritional support, and stress/anxiety reduction has a positive impact on patient outcomes.[1] So far, the focus has mainly been on the fields of surgery[2] and oncology.[3] In surgery, interventions to optimise functional capacity including modifiable risk factors such as anaemia, smoking, and anxiety, as well as exercise programmes to improve cardiovascular function and muscle function, have been shown to improve patient outcomes, with lower postoperative complication rates and earlier restoration of functional status.[2] In oncology, prehabilitation interventions including exercise, nutrition, and addressing psychoeducational aspects of patient care have been shown to improve patient outcomes including cardiopulmonary function, lung function, and mood 30 days post-treatment. This seems to be particularly effective when combined with rehabilitation.[3]

Now is an opportune moment to examine how this concept could be applied in the field of rheumatology, with this article focussing on prehabilitation prior to the commencing of advanced therapies. Included are the biologic disease-modifying antirheumatic drugs (bDMARD) adalimumab, etanercept, certolizumab pegol, golimumab, abatacept, tocilizumab, and rituximab; a targeted synthetic disease-modifying antirheumatic drug (tsDMARD); and the JAK inhibitors (JAKi) tofacitinib, baricitinib, upadacitinib, and filgotinib for rheumatoid arthritis (RA). Adalimumab, etanercept, certolizumab pegol, and tocilizumab are licensed for the treatment of moderate-to-severe RA. Infliximab, golimumab, abatacept, and rituximab are licensed for use in combination with methotrexate for the treatment of moderate-to-severe active RA. Tofacitinib, baricitinib, and upadacitinib are licensed for the treatment of moderate-to-severe RA. The European Medicines Agency (EMA) is currently evaluating filgotinib as a treatment for active RA.

An estimated 60–70% of patients with RA respond to bDMARD,[4] and the newer tsDMARD show similar response rates.[5,6] This leaves 30–40% of patients who do not respond adequately, meaning their inflammatory arthritis remains uncontrolled and requires them to switch to other therapies. One approach to deal with this involves the use of stratified medicine approaches based on the accurate phenotyping of patients with RA using genomic, proteomic, and synovial membrane histological phenotyping. The authors suggest that another way to address the high level of non-responders to treatment involves assessing all modifiable factors that could be improved in advance of therapeutic interventions to maximise the likelihood of success. This article reviews the literature and provides a practical guide to how attending healthcare professionals can optimise patients' chances of responding to advanced therapies using the concept of prehabilliation. This will not only provide patients with the best chance of achieving disease remission, but by using prehabilliation, individuals can be empowered to modify their own behaviours to help treat their disease. Approaches used to support patients' prehabilitation are summarised in Figure 1.

SMOKING

Smoking is associated with an increased risk of structural damage progression in RA, with a cross-sectional study showing that smokers were significantly more likely to have joint space narrowing and erosions ($p<0.05$).[7] Because of this, smoking status should be assessed in all patients and reassessed at regular intervals. Smoking is the most modifiable and predictive factor for TNF inhibitor (TNFi) treatment currently known. Registry data have shown an odds ratio of 0.52 (95% confidence interval [CI]: 0.29–0.96) for good response compared to non-smokers at 3 months,[8] and a prospective study showed greater improvement in disease activity score 28 (DAS28) for non-smokers compared to smokers at 3 months (0.28 greater improvement; 2.64 versus 2.07; $p=0.002$).[9] Past smoking did not affect the response rate, suggesting that if patients can be supported in stopping smoking their likelihood of a good response can revert to that of a non-smoker.[8] Interventions targeting smoking prevention therefore have the potential to improve TNFi response rate, as well as vastly improving the patient's overall health status.

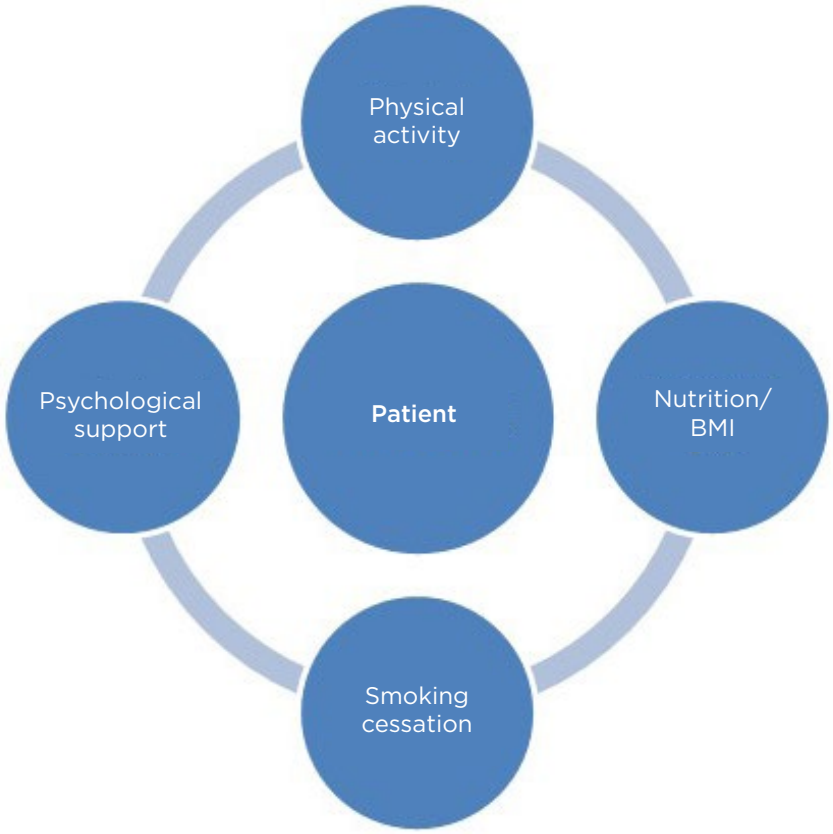

Figure 1: Prehabilitation approaches to support patient.

Smoking status with other bDMARD has been less well studied, but an observational study did not show a difference in response to tocilizumab,[10] and a large multi-national study looking at smoking status and response to rituximab also did not support smoking status as a predictor of response.[11] The effect of abatacept is unknown. JAKi therapies have less available data, but one study has shown that current smokers treated with baricitinib have significantly increased radiographic progression, using the modified total Sharpe score, at 52 weeks (p=0.037).[12] Although, a study pooling data from five Phase III trials including 3,315 patients did not find any significant difference in response to tofacitinib, regardless of baseline smoking status.[13]

All smokers should be offered support to quit smoking prior to commencing advanced therapies, with recent National Institute for Health and Care Excellence (NICE) guidelines including a range of evidence-based interventions, such as behavioural therapy; nicotine replacement; bupropion; a noradrenaline-dopamine reuptake inhibitor, which has been shown to be an effective add-on treatment in smoking cessation; and varenicline, a partial nicotinic acetylcholine receptor agonist.[14]

PHYSICAL INACTIVITY AND POOR FITNESS

Physical exercise has multiple beneficial effects for patients with RA, including improved cardiovascular fitness, improved cognitive function, increased bone density, and reduced fatigue,[15] and all patients should be encouraged to include aerobic and resistance exercise as part of their routine care. Several exercise programmes have also been shown to improve disease activity scores in RA, with no evidence to suggest that increased activity will trigger a disease flare.[15,16] One study found that a short-term intensive exercise programme, involving high-intensity interval walk training, for patients with active RA was more effective in improving muscle strength than a conservative exercise programme.[16] Another study found a reduction in DAS28 of 0.7 in patients undergoing a 10-week

intensive programme compared to the control group who received usual care (DAS28 2.4 versus 3.1; p=0.001).[17] European League Against Rheumatism (EULAR) guidelines recommend that physical activity promotion should be an integral part of the management of patients with inflammatory arthritis and state that healthcare professionals have a responsibility to promote and facilitate physical activity for patients.[18] Sarcopenia is more prevalent amongst RA patients, with approximately 40% of RA patients being defined as sarcopenic (a relative skeletal mass index of <5.5 kg/m^2 for females and <7.26 kg/m^2 for males). Sarcopenic RA patients are more likely to have increased cardiovascular events, as well as progressive erosive changes on X-ray, suggesting that interventions to improve muscle mass could be of benefit.[19] The effect of exercise interventions prior to the initiation of RA treatments and the impact on efficacy has not been widely studied. Although there is evidence that physical exercise improves muscle tone, bone mass, and cognitive function, as well as aiding weight loss, there is currently no evidence that physical activity has a direct effect on an RA patient's response to bDMARD.

NUTRITION AND BMI

An increasing number of patients are overweight (BMI >25), and recent World Health Organization (WHO) data have classified 58.3% of European adult males and 51.2% of European adult females as overweight, with the number predicted to continue rising.[20] Obesity is considered as a mild chronic inflammatory state; adipose tissue produces cytokines such as TNF and IL-6 having proinflammatory properties.[21]

Obese patients with RA have a significantly reduced chance of remission with TNFi treatment, with a meta-analysis showing that the odds of failing TNFi therapy being up to 60% higher in patients with a high BMI,[22] and registry data showing improvements in DAS 28 being 0.22 lower in obese patients receiving TNFi at 6 months (-0.22; 95% CI: -0.42 to -0.03).[23] Obesity did not impact on the efficacy of abatacept or rituximab (cell-targeted therapies).[23,24] For tocilizumab, some studies have shown reduced efficacy in overweight female patients;[23] however, recent data from the American Corrona® RA registry suggest that BMI has no effect on the response to treatment with tocilizumab.[25] Some early real-world data suggest that obesity predicts a poor response to the JAKi baricitinib and tofacitinib, with low disease activity reached by 42% of patients with normal weight, but only by 19% of obese patients, at 6 months.[26] Figure 2 depicts a suggested treatment algorithm for obese patients going onto advanced therapies.

Addressing weight management is challenging. To improve the effectiveness of advanced therapies and reduce the overall disease burden, particularly in patients receiving TNFi medications, consideration needs to be given to achieving an optimum and healthy BMI, ideally before starting the advanced therapy. This can be achieved by having healthy open discussions between the clinicians and patients, including an exploration of their perceptions and motivations. Consideration can also be given to providing support, for example referring patients to dieticians, physiotherapists, support groups, and other services available in the community to help reduce weight.

RA patients are at significantly increased risk for osteoporosis and bone loss because of RA disease processes and glucocorticoid use and RA patients are at increased risk of fragility fractures.[27,28] RA has therefore been incorporated as a dichotomous predictor in the WHO fracture risk assessment (FRAX) algorithm for predicting the 10-year risk of hip or major osteoporotic fracture.[29] RA patients should have their bone health assessed at regular intervals, including vitamin D levels, with appropriate calcium and vitamin D supplementation being prescribed if indicated. The FRAX tool can be used to evaluate 10-year risk of fragility fracture and aid decision-making regarding additional treatments, such as bisphosphonates.

The role of diet and the gut microbiome and the initiation of RA in genetically susceptible patients is of ongoing interest.[30] One study showed that RA patients with higher plasma n-3 polyunsaturated fatty acids levels when starting etanercept had lower DAS 28 scores at 3 months (-0.51; p=0.007). *In vitro* etanercept treatment led to an increase in IL-17 expression, but this increase was not seen in patients with high plasma n-3 polyunsaturated fatty acids levels, suggesting a possible mechanism of action.[31]

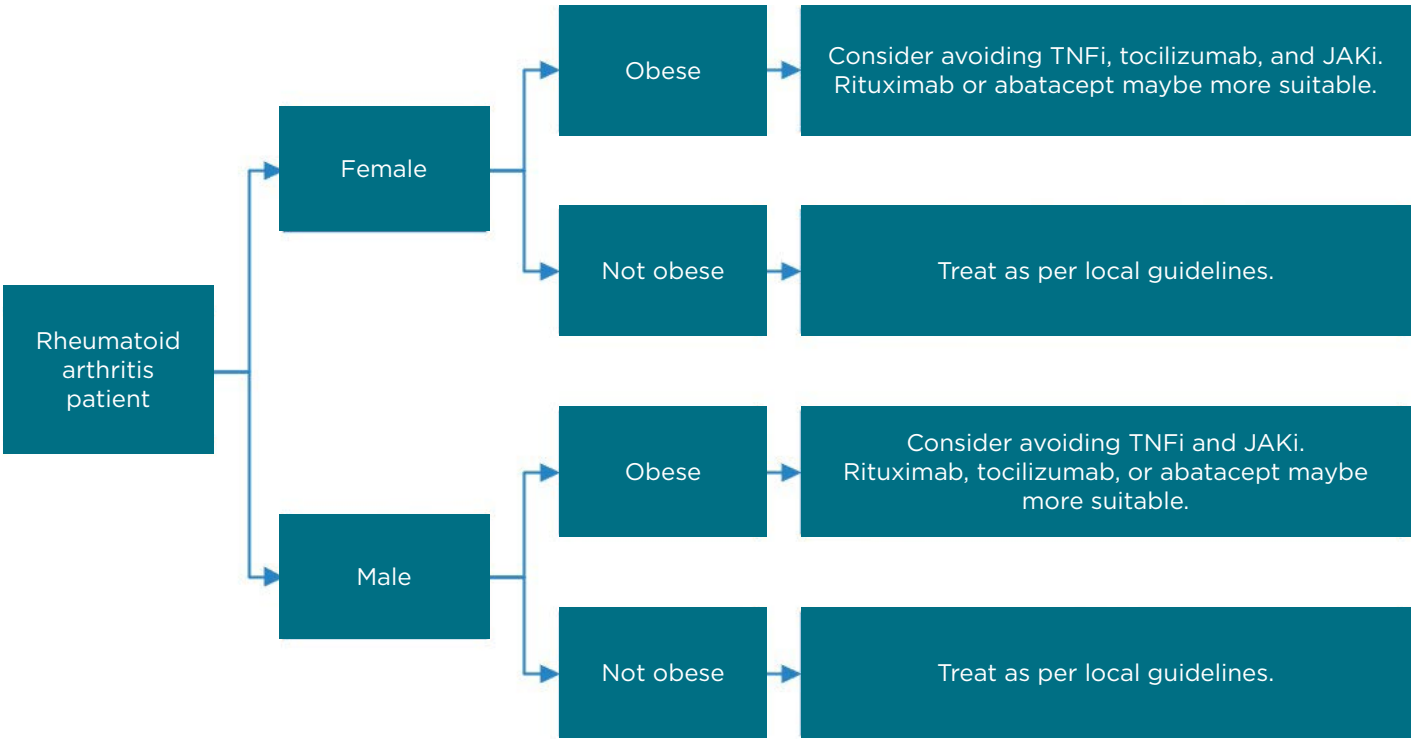

Figure 2: Suggested treatment algorithm for patients going onto advanced therapies for rheumatoid arthritis.

JAKi: JAK inhibitor; TNFi: TNF inhibitor.

A meta-analysis has not shown any conclusive evidence for the use of probiotics in RA.[32] Likewise, no conclusive evidence has been found for a therapeutic effect of dietary fibres and wholegrains, fruit, spices, or of elimination of gluten or meat.[33] Further research is being carried out in this area to look at the effect of dietary supplementation.

Excess sodium is known to increase the differentiation and activation of Th17 cell pathways by inducing serum glucocorticoid kinase 1.[34] Marouen et al.[35] sought to explore this by comparing sodium intake in RA patients with matched controls. Sodium intake was evaluated by 24-hour urinary sodium excretion. Sodium excretion was greater for patients with early RA than controls, even once confounding factors were accounted for (p=0.043). Additionally, patients with radiographic erosion at the time of diagnosis had a higher sodium excretion than those without (p=0.028). Further interventional studies looking at the effect of sodium restriction in the diet on RA disease activity and progression would be of benefit.

PSYCHOLOGICAL SUPPORT

Depression commonly occurs with RA, affecting 13–20% of patients, which is 2- to 3-times higher than the prevalence in the general population.[36] Both health assessment questionnaire (HAQ) score and pain score reporting are strongly influenced by depression, with depressed patients scoring 0.25 HAQ units higher than non-depressed patients and depressed pain giving a visual analogue pain score of 1.9 higher, when scoring from 0 to 10 (p<0.0001).[37,38] Persistent depression is poorly recognised in rheumatology clinics and is associated with a poorer response to TNFi, with one study showing a 0.49 lower improvement in DAS28 at 3 months in patients with depression at onset of treatment compared to those without (DAS28 improvement 1.7 versus 2.2; p=0.005), with the difference observed because of both objective and subjective measures.[39] Other studies have supported this finding,[40,41] although a South Korean health insurance database did not show any difference in TNFi discontinuation rate between patients with depression and controls.[42] There has been

comparatively less research investigating non-TNFi bDMARD, but one study showed that RA patients with elevated plasma IL-6 and IL-17 levels were more likely to have symptoms of depression.[43]

Efforts should also be made to support patients in adhering to their medications. Several studies have shown that patients of all age groups, particularly the young, prefer subcutaneous administration of medication as compared to intravenous administration. Lack of localised skin reaction, reduced frequency of dosing, and fast onset of action are also important to patients.[44] Patient preferences and beliefs have been shown to be important in adherence to medications, with patients considering whether their beliefs about the necessity of the medication outweigh any concerns regarding the potential adverse effects of taking them.[45] Patients concerns and beliefs can be explored using several methods, including the Beliefs about Medicines Questionnaire (BMC),[46] to help improve understanding, allay fears, and ultimately improve adherence. For patients to be able to use subcutaneous injections, they, or a relative or friend, will need to be able to have the manual dexterity to administer the drug and this may be challenging for some patients with chronic RA affecting their hand movements.

A Cochrane review of patient education in RA examined various interventions, including formal structured instruction on RA and ways to manage arthritis symptoms, psychobehavioural methods to promote changes in health behaviours, as well as instructional interventions including exercise, biofeedback, and psychosocial supports. The review showed a small but statistically significant improvement in scores for disability, pain, patient global status, psychological status, and depression.[47] Other meta-analysis reviews support the idea that providing psychological support can be beneficial for patients, with one having shown that cognitive behavioural therapy can significantly reduce levels of anxiety ($p=0.005$) and depression ($p<0.00001$) and relieve fatigue symptoms ($p=0.006$) in RA patients;[48] furthermore, another study showed that mindfulness interventions can reduce depression ($p=0.02$) as well as improve DAS28 scores (-0.29 improvement; 95% CI: -0.38 to -0.19); $p<0.00001$).[49]

DISCUSSION

bDMARD and tsDMARD have revolutionised the treatment of RA; however, there remains a significant number of patients who fail to achieve a good response to treatment. By targeting smoking cessation, physical activity, weight reduction, diet, and providing psychological support, we have the potential to improve the likelihood of patients responding to these drugs. Table 1 summarises different interventions and potential improvements in disease activity scores.

Table 1: Estimated improvements in disease activity score 28 (DAS28) score with prehabilitation intervention.[9,17,23,39]

Intervention	Potential reduction in disease activity score 28 (DAS28)
Smoking cessation	0.28
Exercise programme	0.70
Weight reduction	0.22
Psychological support	0.49

There are a wide range of prehabilitation interventions available to help provide holistic care to patients prior to starting advanced therapies to help maximise a patient's response. Many of these interventions are led by allied health professionals, and physicians would benefit from assessing the frameworks within their healthcare system to address how this multidisciplinary approach is implemented.[50,51] There is good evidence that non-smokers achieve better response rates compared to smokers starting TNFi.[8,9] Further evidence concerning the effect of smoking on JAKi would be of use and future studies and registry data may be able to guide this. Several evidence-based interventions exist, as outlined above, and starting an RA patient on an advanced therapy can provide an opportune moment to promote smoking cessation. Exercise programmes can provide a range of health improvements for RA patients, including cardiovascular capacity, muscle mass, bone density, and disease activity.[15-17] Several exercise programmes have been studied in the literature and it may be of benefit for clinicians to work with allied health professionals locally, including physiotherapists, to help RA patients increase physical activity. Being obese predicts poor response to TNFi,[22] being female and obese possibly predicts poorer response to tocilizumab,[23] and response to JAKi may also be reduced for obese patients.[26] Achieving a healthy BMI has a huge range of health benefits and aiding weight loss can be beneficial for an RA patient's inflammatory arthritis as well as their overall general health. Engaging with patients' general practitioners and local dietetics department may help provide practical assistance in achieving this. In terms of diet, RA patients have a significantly higher risk of osteoporosis and should have their bone health assessed at regular intervals and vitamin D and calcium supplementation should be considered. There is some interesting research looking at diet supplementation with polyunsaturated fatty acids and the effect on disease activity, and it will be of interest to see if further evidence supports their use in the future. Depression is more prevalent amongst RA patients and some studies have suggested that this is associated with a poorer response to TNFi, although this finding has not been replicated in registry data[42] and further studies exploring this would be of use. Providing psychological support, in the forms of cognitive behavioural therapy and mindfulness, have the potential to reduce depression, anxiety, fatigue, and disease activity.[48,49] This suggests that it would be of benefit for departments to identify patients who could gain benefit from psychological support and implement approaches to support them. If a patient is diagnosed with depression, it is also important to involve general practitioners and psychiatry services as appropriate. Finally, exploring patient preferences and beliefs is important in improving adherence to medications to help maximise response.

CONCLUSION

These prehabilitation interventions can improve our patients' cardiovascular health, aerobic function, fatigue symptoms, and quality of life. These different prehabilitation approaches are likely to be interlinked and may in some cases be symbiotic, and it would be of interest to explore the cumulative effect of these approaches. There are many more questions to be answered regarding prehabilliation and it is an exciting area for further research. This article provides a guide to approaches that can be used right now in our clinics when prescribing advanced therapies to give patients the best chance of success.

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Clinical Profile and Outcome of Children with Acute Central Nervous System Infection in Kerala, India

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Disclosure: The authors have declared no conflicts of interest.

Received: 18.04.20

Accepted: 15.06.20

Keywords: Acute central nervous system (CNS) infection, children, clinical profile, encephalitis, meningitis, meningococcus, pneumococcus, scrub typhus.

Citation: EMJ. 2020;5[4]:96-104.

Abstract

Background and aims: Infection of the central nervous system is a significant cause of morbidity and mortality in children. The aim of this study was to evaluate clinical profile and outcome of children aged 1 month to 15 years admitted with acute central nervous system infection between 2008 and 2020 in the Department of Pediatrics, Ananthapuri Hospitals and Research Institute, Thiruvananthapuram, India.

Materials and methods: This was a case record based retrospective study.

Results: Of 62 children, 44 had meningitis and 18 had encephalitis. Most patients were in the age group 1–5 years old and males were the predominant sex (70.96%). Eighteen patients with meningitis had a clinical triad of fever, headache, and vomiting, while only three with encephalitis experienced this. Seizures and altered sensorium were seen significantly more in children with encephalitis. Cerebrospinal fluid pleocytosis was seen in significantly more patients with meningitis compared with patients with encephalitis. Aetiology for meningitis included pneumococcus, *Orientia tsutsugamushi* (scrub typhus), meningococcus, and *Angiostrongylus cantonensis* infection. Causes of encephalitis included enterovirus, mumps virus, herpes simplex virus, dengue virus, and H1N1 influenza virus infection. Paediatric intensive care unit admission was more common for patients with encephalitis. One child with pneumococcal meningitis and another with dengue encephalitis died. Seizures were the most common sequelae.

Conclusion: Typical clinical features were not present in most patients with meningitis; therefore, a high index of suspicion is needed for early diagnosis. Exact aetiologies could not be identified in most of the patients. Pneumococcus, scrub typhus, and meningococcus were the aetiological agents identified for meningitis. Encephalitis was attributed to dengue virus, herpes simplex virus, enterovirus, mumps virus, and H1N1 influenza virus infection.

INTRODUCTION

Infection of the central nervous system (CNS) is a significant cause of morbidity and mortality in children. Depending on the tissues affected, infection could result in meningitis, encephalitis, meningoencephalitis, or a brain abscess.[1] Globally, the incident cases of meningitis increased from 2.50 million (95% uncertainty interval: 2.19–2.91) in 1990 to 2.82 million (95% uncertainty interval: 2.46–3.31) in 2016, but the global number of meningitis deaths was estimated to have decreased by 21% from 403,012 in 1990 to 318,400 in 2016.[2] In India, the incidence and age standardised rate of meningitis have decreased by 51.7% and 28.1%, respectively, from 1990 to 2016.[2] This was achieved by the universal immunisation programmes against major pathogens, such as *Haemophilus influenzae* Type b, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. Acute encephalitis, defined as the acute onset of fever, a change in mental status, and/or new onset of seizures (excluding simple febrile seizures), is clearly a pressing public health emergency in India. Recurrent epidemics of encephalitis of unknown aetiology have occurred in the country.[3]

Kerala, India, experiences a tropical climate, as it is located 800 miles from the equator. [4] The advancements in healthcare in Kerala are on par with developed countries and the country achieves among the best health outcomes with a fairly robust primary healthcare system compared with other states in India. Every village panchayat (village council) has a primary healthcare centre; there is a subcentre for every 5,000 people, which is standard for India. Approximately 60–70% of primary care services are provided in private hospitals. Some patients are referred to hospitals from subcentres, but most people seek care directly from major hospitals.[5] In 2019, there were 59 reported cases and five deaths from acute encephalitis syndrome in Kerala. Of these cases, 11 diagnoses and two deaths were caused by Japanese encephalitis.[6]

This study evaluated the demographic, clinical, and radiological profiles, and outcome of acute CNS infection in children aged 1 month to 15 years admitted to the Department of Pediatrics, Ananthapuri Hospitals and Research Institute in Kerala, India, between 2008 and 2020.

MATERIALS AND METHODS

This was a case record based retrospective study. Case records of all patients aged 1 month to 15 years who were admitted with acute CNS infection to the hospital between 2008 and 2020 were reviewed. Demographic data, clinical features, radiological features, course during hospital stay, and follow-up data were recorded using a structured pro forma. Inclusion criteria were ages 1 month to 15 years, files having complete data, and conclusive diagnosis of acute CNS infection. Case files with incomplete data, noninfective aetiologies, and age group <1 month and >15 years were excluded. The diagnosis of each patient was revised using clinical features and lab evidences. Automated complete blood counts were done using the Coulter principle. Differential counts were also rechecked manually. Absolute neutrophil count (ANC) >10,000 /mm^3 was considered as neutrophilic leukocytosis and absolute lymphocyte count >8,000 /mm^3 was considered as lymphocytic leukocytosis. Erythrocyte sedimentation rate (ESR) was determined using Westergrens principle. C-reactive protein (CRP) was measured using immuno-microslide assays. ESR >40 mm/hr and CRP >30 mg/L were considered positive. Cerebrospinal fluid (CSF) was centrifuged at 3,000 rpm and checked under light microscopy for cytology. CSF gram staining was carried out in all patients. CSF protein and sugar were tested with the turbulometric method. CSF protein >80 mg/dL was considered to be elevated, though age-dependent variations in normal value were present. CSF glucose <45 mg/dL or CSF blood–glucose ratio <0.6 were considered as hypoglycorrhachia. Blood and CSF cultures were performed using the BACTEC™ method (Becton, Dickinson and Company, Franklin, New Jersey, USA). CSF multiplex PCR, brain MRI, or electroencephalogram was carried out for some patients. Reports of radiological investigations were analysed and classified as features of encephalitis, meningitis, or both.

Patients with clinical features of meningitis with ANC >10,000 /mm^3, elevated ESR and CRP, and CSF neutrophilic pleocytosis with or without elevated protein and hypoglycorrhachia were classified as having bacterial meningitis. Patients with CSF gram stain showing bacteria with sterile cultures were also considered

as having bacterial meningitis. Patients with clinical features of meningitis without clear evidence of bacterial meningitis from laboratory findings and who received oral or parenteral antibiotics prior to admission were considered as partially-treated meningitis. Patients with clinical features of meningitis with normal ANC and CSF lymphocytic pleocytosis without prior antibiotic administration were considered as having viral or aseptic meningitis. Any pathogen identified in CSF PCR was considered as the aetiological agent. Patients with hemiplegia and or cranial nerve palsy along with radiological features or laboratory evidence with or without strong contact history were considered as tuberculous meningitis, given the high incidence in India. Patients with fever and altered sensorium with or without seizures and without alternative diagnosis were considered as having encephalitis. Patients with radiological features of encephalitis in MRI brain scans were also included in this group. Telephone interviews with all patients were carried out to record the sequelae of the disease. SPSS® Statistics V22.0 (IBM, Endicott, New York, USA) was used to analyse the results. Chi-square test and Fisher's exact test were used to determine the level of significance. Percentages and averages were also calculated. Institutional ethical committee approval was obtained prior to the study.

RESULTS

Sixty-two children with acute CNS infection were enrolled into the study. Forty-three patients had meningitis, 17 patients had encephalitis, one had meningoencephalitis, and one had cerebral abscess. For the ease of analysis, cerebral abscess was included in meningitis group and meningoencephalitis was included in encephalitis group. There were 27 patients (43.54%) in the age group 1–5 years and 16 patients in the infant (1 month–1 year old) group (25.80%); together, they constituted 69.34% of the study population. There were 44 males (70.96%) enrolled in the study and this was the predominant sex. Similar age and sex distributions were seen in patients with encephalitis or meningitis. Twenty-nine (46.77%) patients received either oral (nine) or parenteral (20) antibiotics before admission. The most common antibiotic used was intravenous ceftriaxone (7) followed by amikacin (5), the most common oral antibiotic used was cefixime (3), and intravenous acyclovir was used in five patients before admission. Comorbidities contributing to acute CNS infection were seen in seven (15.90%) patients with meningitis (Figure 1A and 1B).

There were 18 (40.90%) patients with meningitis who had a clinical triad of fever, headache, and vomiting; three patients (16.66%) with encephalitis experienced this, but the difference was not statistically significant (p=0.082). All patients with encephalitis had fever with any of the neurological symptoms. The most common neurological symptom was seizures experienced by 14 patients (77.77%) followed by altered sensorium experienced by 11 patients (61.11%). Neurological symptoms were present in 21 patients (47.72%) with meningitis. Irritability, experienced by 14 patients (31.8%), was the most common neurological symptom in the meningitis group followed by seizures, experienced by 10 patients (22.72%). Seizures (p<0.001) and altered sensorium (p=0.002) were seen significantly more in children with encephalitis (Table 1).

The most common clinical sign seen in patients with meningitis was neck stiffness (10/44 [22.72%]) followed by low Glasgow Coma Scale (GCS) (9/44 [20.45%]). In patients with encephalitis, the most common clinical sign was low GCS (10/18 [55.55%]) followed by motor abnormalities (4/18 [22.22%]). Of all the clinical signs, low GCS was seen significantly more in children with encephalitis (10/18; p=0.006) (Table 1). ANC >10,000 /mm^3 was seen in 19 (43.18%) patients with meningitis and seven (38.88%) patients with encephalitis. Peripheral eosinophilia was seen in one patient with eosinophilic meningitis. Elevated CRP was more significant in patients with meningitis compared with encephalitis (p=0.025). Blood culture was sterile in all patients (Table 1).

CSF pleocytosis was seen in 41 (93.18%) patients with meningitis compared with nine (50%) patients with encephalitis (p<0.001). Most patients with meningitis had lymphocytic pleocytosis in CSF (35/44 [79.55%]). Neutrophilic pleocytosis was only seen in six (13.63%) patients. CSF protein levels were elevated in 14 (31.81%) patients with meningitis and one patient with encephalitis (p=0.049) (Table 1).

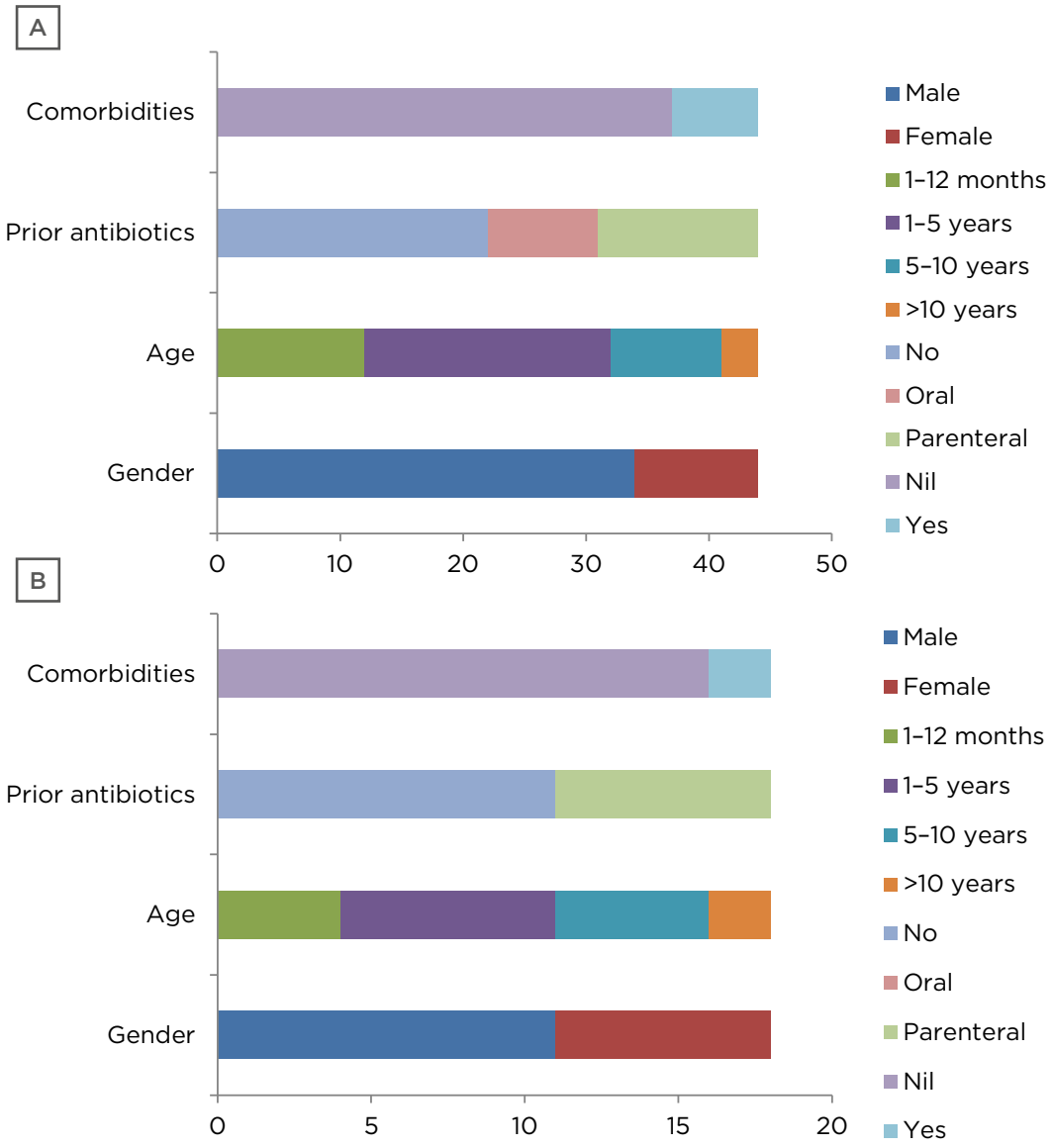

Figure 1: Demographic profile of **A)** meningitis group and **B)** encephalitis group.

A CSF gram stain showed gram positive cocci in two patients, but the culture was sterile. The CSF culture grew penicillin-sensitive *S. pneumoniae* in one patient. CSF PCR showed *S. pneumoniae* (one patient), *N. meningitidis* (one patient), *Angiostrongylus cantonensis* (one patient), enterovirus (one patient), and herpes simplex virus (one patient).

MRI of the brain was completed for 34/62 (59.67%) patients, 17 (50%) of whom had normal results. Eight patients with meningitis and nine patients with encephalitis had positive MRI findings (p=0.008) (Table 1). Two patients with meningitis had basal ganglia involvement and one had mumps encephalitis. One patient with encephalitis was found to have acute necrotising encephalopathy of childhood caused by H1N1 influenza virus infection. One patient had multiple cerebral abscesses. Electroencephalogram was abnormal in 15 (83.33%) patients with encephalitis and two (11.11%) patients experienced nonconvulsive status epilepticus.

Twenty-six (59.09%) patients with meningitis and 17 (94.40%) patients with encephalitis were admitted to the paediatric intensive care unit (p=0.012). Five (8.06%) patients required invasive ventilation.

Table 1: Clinical features and investigations of the study population.

	Meningitis (n=44)		Encephalitis (n=18)	
	n	Percentage (%)	n	Percentage (%)
Triad of fever, headache, and vomiting	18	40.90	3	16.67
Seizures*	10	22.72	14	77.78
Irritability	14	31.80	6	33.33
GCS <15*	9	20.45	10	55.56
Motor abnormalities	4	9.09	4	22.22
Cranial nerve palsy	1	2.27	0	0.00
Meningeal signs	10	22.72	2	11.11
Bulging AF	3	6.81	0	0.00
Blood count				
Normal	15	34.09	9	50.00
Neutrophilic leukocytosis (ANC >10,000 /mm^3)	19	43.18	7	38.89
Lymphocytic leukocytosis (ALC >8,000 /mm^3)	9	20.45	2	11.11
Eosinophilia	1	2.27	0	0.00
ESR				
Negative	25	56.81	16	88.89
>40	15	34.09	2	11.11
>100	4	9.09	0	0.00
CRP				
Negative <30	30	68.18	16	88.89
Positive >30*	14	31.81	2	11.11
CSF cytology*				
Normal	2	4.54	9	50.00
Neutrophilic pleocytosis	6	13.63	2	11.11
Lymphocytic pleocytosis	35	79.55	7	38.89
CSF protein				
Normal	30	68.18	17	94.44
Increased*	14	31.81	1	5.56
Hypoglycorrhachia				
Present	25	56.81	7	38.89
Absent	19	43.18	11	61.11
Brain MRI				
Not done	25	56.81	3	16.67
Positive*	8	18.18	9	50.00
Negative	11	25.00	6	33.33

*p<0.05

AF: anterior fontanelle; ANC: absolute neutrophil count; ALC: absolute lymphocyte count; CRP: C-reactive protein; CSF: cerebrospinal fluid; GSC: Glasgow Coma Scale; ESR: erythrocyte sedimentation rate.

For most patients (77.28% with meningitis and 94.44% with encephalitis), first-line antibiotics, such as ceftriaxone or cefotaxime with vancomycin, were started at admission. In other patients, second-line antibiotics, such as piperacillin/tazobactam or meropenem, were used. Antibiotics were upgraded for seven patients with meningitis and for two patients with encephalitis. Acyclovir was used empirically in 20 (45.45%) patients with meningitis and all patients with encephalitis. Other treatments given in both groups are shown in Table 2. Mean duration of antibiotic treatment was 8.18 days for meningitis and 7.44 days for encephalitis, and mean duration of hospital stay was 11.97 and 19.83 days, respectively.

Exact aetiology was only identified in 11 (17.7%) patients. Six (13.95%) patients with meningitis had acute bacterial meningitis and 18 (41.86%) had partially-treated meningitis; eight (18.60%) patients had viral meningitis. A diagnosis of tuberculous meningitis was made for four patients based on clinical features, including cranial nerve palsy or hemiplegia; radiological features, such as basal meningitis with vasculitis; and contact history; evidence of tuberculous infection could not be obtained. The patients were treated with antituberculous drugs. Two children had pneumococcal meningitis and they were both >5 years old. One child had ventriculoperitoneal shunt *in situ* and survived. The other child had fulminant presentation of septic shock and cardiac dysfunction. Other aetiological agents identified are given in Table 2.

Table 2: Treatment of study population.

	Meningitis (n=44)		**Encephalitis (n=18)**	
	n	Percentage (%)	n	Percentage (%)
PICU admission*	26	59.09	17	94.44
Initial antibiotics				
First-line antibiotics	34	77.27	17	94.44
Second-line antibiotics	10	22.72	1	5.56
Upgradation of antibiotics	7	15.90	2	11.11
Steroids	9	20.45	5	27.78
Acyclovir	20	45.45	18	100.00
Antiepileptics	10	22.72	17	94.44
Mannitol	10	22.72	4	22.22
Invasive ventilation	2	4.54	2	11.11
Antituberculous treatment	4	9.09	0	0.00
IVIG	0	0.00	2	11.11
Plasmapheresis	0	0.00	1	5.56
Oseltamivir	1	2.27	1	5.56

*$p<0.05$

IVIG: intravenous immunoglobulin; PICU: paediatric intensive care unit.

Complications were seen in eight (12.90%) patients. Septic shock was the most common complication (three patients), followed by acute kidney injury (two patients), nonconvulsive status epilepticus (two patients), catheter-related urinary tract infection (two patients), aspiration pneumonia (two patients), status epilepticus (one patient), and cardiac dysfunction (one patient). Two (3.22%) patients died as a result of acute CNS infection: one had pneumococcal meningitis with multiorgan dysfunction and the other patient had dengue encephalitis with super refractory seizures. Overall case fatality rates for acute CNS infection in this study was 3.2%, 2.3% for meningitis, and 5.5% for encephalitis.

Follow-up over the telephone was completed for 39 patients. Seven patients (38.88%) with encephalitis had sequelae. Seizures needing continuation of antiepileptic medications was the most common sequelae and was required by four patients, and poor scholastic performance was reported in two patients. Other sequelae reported included developmental delay, aphasia, motor abnormalities, and dystonia. One patient with meningitis had hearing difficulties.

DISCUSSION

In this study, most children were <5 years of age (70.96%). Males were the predominant sex in the study group (70.96%) and the male–female ratio was 2.44. In a study by Debnath et al.[7] in Pune, India, in children <12 years of age, 74.68% were <5 years of age; 46.8% were infants, 27.9% were 1–5 years old, and male–female ratio was 1.82.[7] In a similar study by Mani et al.[8] from 1996 to 2005, the male–female ratio was 3.18.[8]

Typical clinical features were not present in all children. In this study, less than one-half of patients with meningitis had the triad of fever, headache, and vomiting. Only approximately one-half of the patients had neurological symptoms. Irritability was the most common neurological symptom in patients with meningitis. A similar demographic was seen in a study by Chauhan et al.[9] In the 2009 systematic review by Curtis et al.,[10] a lack of irritability lowered the odds of the meningitis by one-half, but the presence of irritability did not strongly signify the presence of meningitis.[10] Only 25.5% of patients with meningitis had meningeal signs, similar to results seen in the study by Chinchankar et al.[11] A high index of suspicion is needed to identify children with meningitis early. All patients with encephalitis had fever with neurological symptoms, seizures being the most common. More seizures and altered sensorium were observed in patients with encephalitis, which was expected. In a study by Tripathy et al.,[12] low GCS was more significantly observed in patients with viral encephalitis compared with nonviral cases.[12]

Elevated CRP, CSF pleocytosis, and elevated CSF protein were significantly higher in patients with meningitis. In the study by Fitzwater et al.,[13] elevated CSF white blood cell counts and protein levels were significantly seen more often in patients with bacterial meningitis than in aseptic meningitis.[13]

Pneumococcus sensitive to penicillin grew in CSF culture for one (1.6%) patient. In a study by Chauhan et al.,[9] CSF culture positivity was seen in 2.0% of patients.[9] Higher CSF culture positivity can be seen in other studies, such as those by Debnath et al.,[7] Mani et al.,[8] and De et al.[14] The low CSF culture positivity seen in this study could be attributed to the approximate one-half of patients having received antibiotics prior to hospital admission, as seen in the study by De et al.[14] CSF PCR was only completed for 28 (45.16%) patients, due to financial constraints. Antigen detection tests were not done in this study, which led to failures in exact aetiological diagnosis in many patients.

Admission to the paediatric intensive care unit was needed significantly more by patients with encephalitis. There were only four patients who needed invasive ventilation, and all were for poor sensorium; most of these patients responded to first-line antibiotics. Second-line antibiotics were given at admission in patients with fulminant presentation and for those who were treated with first-line antibiotics externally. Acyclovir was started empirically in approximately one-half of the patients with meningitis because of an atypical CSF picture and low culture positivity rate.

In India, vaccination against *H. influenzae* Type b has been part of the national immunisation schedule since 2011. Pneumococcal vaccination is part of national immunisation schedule in some states in India. In Kerala, the authors' state,

it is not part of the government immunisation schedule but is available privately. In the Indian Council of Medical Research (ICMR) study by Jayaraman et al.[15] in 2012–2013, the most common aetiological agents for meningitis were *S. pneumoniae* (82.9%), *H. influenzae* Type b (14.4%), and *N. meningitidis* (2.7%). In this present study, two children had pneumococcal meningitis and one with fulminant presentation died on the second day after admission to hospital. This emphasises the importance of pneumococcal vaccination in children. One child with meningococcal meningitis survived without sequelae.

Scrub typhus IgM was positive for two patients with meningitis who had persistent fever. Scrub typhus meningitis, a disease endemic to this part of India, is an important cause of acute and subacute meningitis. Characteristic eschar is seen in some children.[16] CSF findings include mild pleocytosis, mildly elevated protein, and normal glucose.[17,18] In a study by Dinesh Kumar et al.,[19] the incidence of meningoencephalitis was 5% in children with scrub typhus and was commonly seen in ages 5–12 years old, presenting late in the second week of illness during the post-monsoon months. Most of the children responded well to doxycycline.[19]

In this present study, one patient had eosinophilic meningitis, which is a rare entity, and was shown to be caused by *A. cantonensis*. It is characterised by CSF eosinophilia, mildly elevated protein, and normal glucose.[20] This patient had exposure to snails at home, had peripheral as well as CSF eosinophilia, and typical CSF findings. CSF PCR was positive for *A. cantonensis*. The patient was treated with steroids and albendazole, and recovered without complications.

While most studies across 1975–1999 identified the Japanese encephalitis virus as the main cause of acute encephalitis syndrome, many studies published after the year 2000 identified *Chandipura vesiculovirus* and enteroviruses as the most common agents for the syndrome, in both outbreak and surveillance studies.[21] The most common aetiological agent of viral encephalitis in the study by Beig et al.[22] was enterovirus 71, which occurred in 42.1% of cases, followed by measles in 21.1%, varicella-zoster virus in 15.8%, herpes simplex virus in 10.5%, and mumps in 10.5% of cases; Japanese encephalitis virus was not found in any case.[22] In a study by Tripathy et al.,[12] herpes simplex virus was the most common cause of viral encephalitis.[12] In a study by Kumar et al.,[23] herpes simplex virus (31.50%) was the most common virus followed by adenovirus (10.95%), parvovirus (2.73%), Japanese encephalitis virus (1.36%), enterovirus (1.36%), and Epstein–Barr virus (1.36%).

In this study, exact aetiology was identified in only five of the 18 patients with encephalitis. Japanese encephalitis virus was not identified. One child with dengue encephalitis had super refractory seizures and died. Other aetiologies were herpes simplex virus, enterovirus, mumps virus, and H1N1 influenza virus.

Overall, case fatality rate for acute CNS infection in this study was 3.2%; case fatality rate was 2.3% for meningitis and 5.5% for encephalitis. Higher case fatality rate is seen in similar studies.[7,11] In this study, the low rate may be attributable to the small sample size. Almost similar complications and neurological sequelae are seen in similar studies.[7,11]

There are several limitations for this study, which include small sample size and the retrospective nature of the study. Audiology evaluation was not done in most patients, hence data on hearing loss was not available. The hospital is a tertiary care referral centre and patients were from neighbouring districts and states with many seeing nearby pediatricians or neurologists for follow-up; therefore, follow-up data after discharge were not available for all patients and only telephone interviews with the parents were possible. As this hospital is frequented by people with middle and high socioeconomic status, the results may not represent the general population. Lack of antigen detection studies and CSF PCR for all children affected the aetiological diagnoses.

CONCLUSION

Children <5 years of age and male sex were predominantly affected in this study. Typical clinical features were not present in most patients with meningitis; therefore, a high index of suspicion is needed for early diagnosis. Seizures, altered sensorium, and need for intensive care

admission were observed in greater numbers in patients with encephalitis. Elevated CRP, CSF pleocytosis, and elevated CSF protein levels were significantly higher in patients with meningitis compared with patients with encephalitis. For most patients, the aetiological agent could not be identified. Pneumococcus, scrub typhus, and meningococcus were aetiological agents identified for meningitis. Aetiological agents identified for encephalitis were enterovirus, herpes simplex virus, mumps virus, and H1N1 influenza virus. One child died from pneumococcal meningitis and one child died from dengue encephalitis. The most common postencephalitis complications included seizures requiring continued antiepileptic medications.

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Shionogi Europe, 33 Kingsway, London WC2B 6UF, UK, Tel: +44 203 053 4190, email: contact@shionogi.eu

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PP-EU-LUS-0028. Date of preparation: July 2020.

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Prolonged Intraoperative Cardiac Arrest in a Young Patient with Successful Precordial Thump

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Disclosure: The authors have declared no conflicts of interest.

Received: 02.02.20

Accepted: 30.03.20

Keywords: Anaesthesia, cardiac arrest, cardiopulmonary resuscitation (CPR), intraoperative, postresuscitation care, pulmonary embolism (PE).

Citation: EMJ Cardiol. 2020;DOI/10.33590/emjcardiol/20-00024

Abstract

Cardiac arrest during surgery is rare but is one of the most dreaded complications. Precordial thump (PT) had been used for a long time, but in the present day it has become obsolete. In regard to the witnessed onset of asystole, there is insufficient evidence to recommend for or against the use of the PT. This case report is of a 17-year-old male who presented to hospital with a congenital haemangioma on the right calf. He had no other significant medical conditions and was on no other medications. The patient history, clinical examination, and investigations were normal. He had undergone an operation 3 weeks previously where a section of his haemangioma was excised, and an appointment was made for excision of the remaining haemangioma. Anaesthesia induction and endotracheal intubation were smooth and uneventful. Following lifting and exsanguination of the patient's leg by Esmarch bandage, he developed ventricular fibrillation and arrested with asystole. Cardiopulmonary resuscitation was performed, with no good response, for approximately 50 minutes. Lastly, a PT was performed, and the patient's heart rate immediately returned. The operation was postponed. Postresuscitation care was conducted in an intensive care unit. The patient was later discharged without complications.

INTRODUCTION

Cardiac arrest during surgery is rare but is one of the most dreaded complications, particularly when there is no obvious underlying cause or risk. Some studies conducted between 2001 and 2012 quoted the incidence of intraoperative cardiac arrest (ICA) to be 1.10–7.22 per 10,000 surgeries.[1-4] Cardiac arrests related to anaesthesia are classified as avoidable but are thought to have a higher survival rate compared to other possible causes of cardiac arrest in the operating room. Anaesthesia-attributable cardiac arrests are related to airway management and medication administration, which are important considerations in prevention strategies.[2,3] Post-cardiac arrest care using a high-quality integrated multisystem of support and management could significantly affect patient outcome. Post-cardiac arrest care should be implemented according to the 2015 American Heart Association Guidelines Update for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care (AHA CPR ECC).[5]

A 17-year-old male presented to the hospital with a congenital haemangioma on the right calf, measuring approximately 9x10 cm. His body weight was 60 kg. The patient had no other complaints and no relevant medical history. He had a heart rate of 80/minute, respiratory rate of 17/minute, blood pressure of 120/70, and oxygen saturation (SpO_2) of 98%. Clinical examination of the cardiovascular, respiratory, and nervous systems was normal. He had undergone an operation 3 weeks previously where a section of his haemangioma was excised, and an appointment was made for excision of the remaining haemangioma. The investigations, including complete blood count, random blood sugar, liver function test, urea, creatinine, and electrolytes, were all within normal limits. The patient fasted for 8 hours prior to the operation, as per hospital protocol, with intravenous fluids maintenance. The patient was connected to standard monitoring as per the American Society of Anesthesiologists (ASA) guidelines.[6] After preoxygenation with 100% O_2, anaesthesia was induced with 100 µg fentanyl, 130 mg propofol, and 35 mg rocuronium. The tracheal intubation was performed uneventfully. Anaesthesia was maintained with 50% O_2 in N_2O, with an isoflurane minimum anaesthetic concentration of 1.6%. All patient and ventilator parameters were normal. The patient's leg was lifted by the surgeon when applying the Esmarch bandage. Immediately following exsanguinations and compression over both the calf and the site of the previous operation, the patient developed ventricular fibrillation (VF) and became hypotensive and arrested in asystole. Effective cardiopulmonary resuscitation (CPR) was started with 100% fraction of inspired oxygen, bag valve mask ventilation, and cardiac compressions. Checking of the equipment, machines, and breathing system, as well as verification of the endotracheal tube, were completed with no defects reported. Hypoglycaemia was ruled out. Adrenaline was given intravenously at 1 mg every 3–5 minutes, up to a total dose of 10 mg. Intravenous 300 and 150 mg amiodarone were administered. There was no good response for approximately 50 minutes.

The reversible causes of cardiac arrest ('four Hs and four Ts') were checked simultaneously alongside the ongoing effective CPR. Lastly, a precordial thump (PT) was delivered by a senior anaesthetist, and the patient's heart rate immediately returned. With a firmly clenched right fist, at approximately 20 cm above the patient, a strike was given on the inferior third of the patient sternum. The ECG showed a narrow QRS complex tachycardia which then changed to a sinus rhythm. The end-tidal CO_2 on the capnograph was raised immediately. The operation was postponed. The patient was stabilised and transferred to an intensive care unit (ICU). Monitoring was continued, and an arterial line was inserted for invasive blood pressure and repeated arterial blood gases sampling. Synchronised intermittent mechanical ventilation mode was used. Four hours later, the patient recovered his spontaneous respiration and his level of consciousness began to improve. Two hours after this he was extubated safely.

A pulmonary embolism (PE) that migrated from the location of the previous surgery was thought to be the most likely diagnosis. He was kept in the ICU on 5,000 U heparin intravenous bolus, followed by a continuous infusion of 1,200 U/hour until the following day. On Day 2, he was discharged to the intermediate care unit where he stayed for 2 days. The cardiology, haematology, respiratory, and neurology evaluations, examinations, and investigations were completed and revealed no abnormality, apart from a small thrombus at the site of previous surgery, identified by a doppler ultrasound.

DISCUSSION

Intraoperative blood loss, as indicated by the volume of transfusion, was the most important predictor of ICA. ASA status and functional status were other important risk factors.[1-4] Cardiac arrests entirely related to anaesthesia are classified as avoidable and are thought to have a higher survival rate compared to other possible causes of cardiac arrest in the operating room. The study by Braz et al.[3] concluded that all anaesthesia-attributable cardiac arrests were related to airway management and medication administration, which are both important considerations in prevention strategies.[2,3] During surgery, the context of positioning and specific procedures may have a particular effect on cardiac arrest. However, a proper understanding of the mechanisms and management options related to these events

would certainly assist in minimising adverse outcomes.[7] The outcome of cardiac arrest during anaesthesia is generally good, with most patients leaving the hospital alive and seemingly well. A complete systematic assessment of the patient, equipment, and drugs should be completed during an ICA, even if the cause of the cardiac arrest is already hypothesised.[8] Cardiac arrests during nonregular working hours tend to have worse outcomes, which may indicate that the availability of human resources influences survival.[4]

Deep venous thrombosis, in most reported cases, involves the lower extremities. PE can occur in young patients with unknown risk factors and be without anticipation. Schonauer et al.[9] reported a large PE in a 23-year-old woman following bilateral breast augmentation. The authors recommended more restrictive risk assessment scores, such as the Caprini score, because of a possible higher risk of complications secondary to anticoagulant treatment such as bleeding and haematoma.[9] Anticoagulation is the standard for management of PE. The addition of aggressive treatment such as thrombolysis or surgical embolectomy may be needed when the clot burden or clinical presentation warrant such measures Improvement in forward flow and a reduction in right ventricle afterload, to avoid right ventricular failure, is the goal of therapy of large PE. Reducing the degree of occlusion in the pulmonary vasculature and minimising the humoral vasoconstrictors that emanate from intravascular thrombi (mainly serotonin and histamine, produced by neutrophils, endothelium, and platelets), results in a fall in pulmonary vascular resistance and pulmonary artery pressure.[10]

PT had been used for a long time but in the present day it has become obsolete. For unwitnessed out-of-hospital cardiac arrest, the PT should not be used (Class III, Levels of Evidence C). It is not recommended as first-line treatment for VF, but is used in cases in which there is no defibrillator available. It is not effective in terminating VF and may cause rhythm deterioration. For witnessed onset of asystole, there is insufficient evidence to recommend for or against the use of the PT.[11,12] Few studies have described the value of PT as first-line treatment to monitored out-of-hospital cardiac arrest as a result of VF and pulseless ventricular tachycardia.[13] A study by Madias et al.[14] recommended PT for asystole.[14] A serial PT was successfully used in a 28-day-old newborn baby who presented with a refractory supraventricular tachycardia and cardiovascular collapse after amiodarone administration.[15] Post-cardiac arrest care using a high-quality integrated multisystem of support and management could significantly affect patient outcome. Post-cardiac arrest should be implemented following the 2015 AHA CPR ECC.[5] In the patients undergoing valve surgery, measuring postoperative high-sensitivity troponin T is recommended because its elevation is associated with a high risk of postoperative, sudden cardiac arrest.[16] The most likely cause of cardiac arrest in this patient was migration of an embolism from the site of previous surgery. This diagnosis was made based on clinical judgement, as well as the finding of the small emboli on the doppler ultrasound. PE was not ruled out during the cardiac arrest by echocardiogram due to its unavailability at the time. In ICU, the patient was stable, so no further investigations were requested by the cardiology or pulmonology staff.

CONCLUSION

ICA in a young, healthy patient is a feared event. However, anaesthetists should have the capability to deal with such situation. Early recognition, initiation of effective CPR, and established postresuscitation care allow the maximum potential for a good outcome. PT may still have a role in witnessed cardiac asystole.

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Longitudinal Characterisation of the Gastrointestinal Tract Microbiome in Systemic Sclerosis

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Disclosure: The authors have declared no conflicts of interest.

Acknowledgements: The authors would like to thank the patient volunteers for their participation in this study. This work was supported by grants from CURE: Digestive Disease Research Center supported by National Institiute of Health (NIH) grant P30DK41301- Pilot and Feasibility (ERV); NIH PO-1 DK46763 (JB) and UL1TR001881 (JB); and VA Career Development Award IK2CX001717 (JPJ).

Received: 24.02.20

Accepted: 01.10.20

Keywords: Systemic sclerosis (SSc), microbiota, dysbiosis, gastrointestinal tract (GIT).

Citation: EMJ. 2020;5[4]:110-118.

Abstract

Objectives: To evaluate changes in microbial composition and the evolution of gastrointestinal tract (GIT) symptoms in systemic sclerosis (SSc).

Methods: Adult SSc patients provided stool specimens every 3 months over the course of 1 year. Participants completed the University of California, Los Angeles (UCLA) GIT 2.0 questionnaire to assess GIT symptom severity at each stool collection. The microbiota from these samples were determined by Illumina HiSeq 2500 16S ribosomal RNA sequencing (Illumina, Inc., San Diego, California, USA). Mixed effect models evaluated changes in GIT symptoms and microbial composition over time.

Results: Among 19 patients with SSc (female; 89.5%; median age: 51.3 years), the median disease duration was 7 years and the baseline total GIT 2.0 score was 0.7 (standard deviation: 0.6). The majority of participants (63%) provided at least four stool samples over the course of the 12-month study. Patients with longer disease durations had increased GIT symptoms over the course of the

study. There was no difference in the course of GIT symptoms over time between patients with limited versus diffuse cutaneous disease. The relative abundances of specific genera did not change over time within individual subjects. After controlling for age, sex, ethnicity, disease duration, and SSc subtype (i.e., limited versus diffuse), low abundance of *Bacteroides* was associated with increased GIT symptoms over time.

Conclusion: This study is the first to have longitudinally characterised the lower GIT microbiome in SSc patients and demonstrated relative stability of genera abundance over the course of 1 year. The findings provide additional evidence that specific genera are associated with SSc-GIT symptoms and warrant further evaluation in larger SSc studies.

INTRODUCTION

Understanding the interplay between the immune system and gastrointestinal tract (GIT) microbiota is an evolving area of research.[1,2] Emerging evidence suggests that GIT microbiota affects immune function in autoimmune diseases such as inflammatory bowel disease[3] and rheumatoid arthritis.[4]

The authors' groups and several others have discovered alterations in the GIT microbiota (i.e., dysbiosis) within distinct systemic sclerosis (SSc) cohorts.[5-11] For instance, the SSc disease state is associated with increases in pathobiont organisms (e.g., *Fusobacterium*[5,6] and *Akkermansia*[5,6]), and decreases in commensal organisms (e.g., *Faecalibacterium,*[5-7] *Bacteroides,*[6] and *Clostridium*[5,6] genera). The precise immunological implications of these changes in SSc are unclear; however, studies have demonstrated that commensal organisms, such as *Bacteroides fragilis*, induce and maintain regulatory T cells.[12] *B. fragilis* has been found to modulate immune cell responses through the secretion of outer membrane vesicles.[13]

In the authors' prior work, they found that a decreased abundance of *B. fragilis* was associated with increased SSc-GIT symptoms.[5] They also discovered that increased abundance of the pathobiont *Fusobacterium* was associated with increased GIT symptoms.[5] Symptoms of lower SSc-GIT dysfunction include constipation, abdominal pain, diarrhoea, faecal incontinence, and/or weight loss,[14] which all have the potential to contribute to psychosocial dysfunction and poor quality of life.[15,16]

However, the aforementioned studies were cross-sectional; therefore, it is unclear whether the relationships observed between specific genera and GIT symptoms are causational and/or persist with time. To address these questions, the present study sought to longitudinally characterise the GIT microbiome in SSc patients over a 12-month period while simultaneously evaluating GIT symptoms using a valid patient-reported outcome.

The objectives of this study, not previously explored in the SSc literature, were to: 1) evaluate changes in GIT microbiota of SSc patients over time; 2) assess changes in GIT symptoms in SSc patients over time; and 3) evaluate the hypothesis that specific microbial genera are associated with the characteristic symptoms of lower GIT dysfunction in SSc over time.

METHODS

Study Participants

Patient participants were consecutively enrolled from University of California, Los Angeles (UCLA), Los Angeles, California, USA, rheumatology clinics. Eligible participants included adult patients (≥18 years) with SSc.[17] Exclusion criteria included inflammatory bowel disease and the inability to withstand from taking an antibiotic and a probiotic for at least 3 weeks prior to the stool collection. Patients were allowed to continue their proton-pump inhibitor use because this medication appears to exert negligible effects on colonic microbiota,[18] and abstaining from proton-pump inhibitor use in SSc patients can cause undue harm. As this was a proof of concept study to understand the dynamics of microbial composition in SSc patients over 1 year, there was no target sample size. The UCLA Institutional Review Board approved the study protocol and written informed consent was obtained from each participant.

Specimen Procurement and Processing

Participants collected stool specimens every 3 months for 12 months, using the authors' previously published collection method,[19] and the samples were immediately frozen. Frozen specimens were subsequently transferred on ice to the participant's study centre. The study coordinator confirmed that specimens were still frozen at the time of arrival and immediately stored them at -80°C. Please see the authors' previous publication[5] for complete details of specimen procurement and processing.

16S Ribosomal RNA Gene Sequencing and Microbial Composition Analysis

The microbiota from the stool specimens were profiled by multiplex sequencing for bacterial 16S ribosomal RNA genes using an Illumina HiSeq 2500 (Illumina, Inc., San Diego, California, USA). All samples were analysed simultaneously to avoid any batch effects. The exact details of this approach have been outlined in our prior publication.[5]

To compare the microbial communities of SSc samples within each subject, measured every 3 months, alpha and beta diversity analysis were performed in QIIME™. Alpha diversity was assessed by the Chao1 index (metric of species richness) and Shannon index (metric of both richness and evenness). Repeated measures analysis of variance (ANOVA) was used to determine the significance of changes in alpha diversity within each subject. Beta diversity represents differences in microbial composition between samples and was measured using a robust Aitchison distance metric calculated using the DEICODE plugin in QIIME. Principal coordinates analysis was performed to visualise the resulting distance matrix. Significance was determined by permutational multivariate analysis of variance using the Adonis function in the R vegan package.

Assessment of Gastronintestinal Tract Symptoms

On the day of their stool collection, the SSc participants completed the UCLA GIT 2.0 questionnaire, a valid measure of GIT symptom severity in patients with SSc.[20] The questionnaire consists of seven domains and has been validated in several independent SSc cohorts.

Statistical and Bioinformatics Analyses

Analyses were performed using R version 3.1.2. Mean and standard deviation (SD) were used to describe continuous parametric data and median and interquartile ranges were used to describe continuous nonparametric data. All tests were 2-sided with a 0.05 alpha level. The false discovery rate (FDR) correction method of Benjamini and Hochberg[21] was used and a significant association was defined at the FDR q value ≤0.1.

To determine which baseline characteristics were associated with the course of GIT symptoms, each of the following variables were entered one at a time into the mixed models for the longitudinal assessment of GIT symptoms: age, sex, race, ethnicity, diffuse versus limited cutaneous disease, disease duration (years), Scl-70 positive versus negative, anticentromere antibody positive versus negative, presence or absence of interstitial lung disease (ILD) based on high-resolution CT, current prednisone use, and current other immunosuppression use. Among these variables, only disease duration and the presence of limited versus diffuse cutaneous disease were associated with the outcome. Subsequently, both of these variables (i.e., disease duration and the presence of limited versus diffuse cutaneous disease) were simultaneously included in the mixed effects model analysis for the total GIT symptom score and each individual domain score.

Mixed effects models were generated for each individual genus with each patient as a random effect to determine whether the relative abundance of specific genera changed over time. As some genera were present/absent in different patients, we also generated mixed effects models for longitudinal dichotomous data via GLIMMIX, where the outcome was the presence/absence of a particular genus.

Because neither the abundance, nor the presence/absence of any genera significantly changed over time, the average genus abundance level across all stool collections for each patient was calculated. The average abundances were included as a covariate into a rank regression analysis with the GIT score as the outcome. The other covariates in the model were disease duration and limited versus

cutaneous disease. The clinical variables were first entered into the model before entering the genus abundances. This analysis was performed for each individual genus, and the results were adjusted for multiple hypothesis testing.

RESULTS

Participant Characteristics

A total of 19 patients with SSc (female: 89.5%) provided baseline stool samples and completed the UCLA GIT 2.0 questionnaire. The median age was 51.3 years and the median disease duration was 7.0 years (Table 1).

Table 1: Demographic and disease-related characteristics of systemic sclerosis participants.

	SSc participants (N=19)
Age (years)	Median: 51.3 (IR: 48.7–59.4)
Female	17 (89.5%)
Ethnicity	
White	11 (57.9%)
Asian	2 (10.5%)
More than one race	4 (21.1%)
Hispanic	7 (36.8%)
Other	2 (10.5%)
Diffuse cutaneous disease	6 (31.6%)
SSc disease duration (years)	Median: 7.0 (IR: 5.0–16.0)
ANA positive	17/18 (94.4%)
Scl-70 positive	3/13 (23.1%)
Anti-centromere positive	5/13 (38.5%)
HRCT-defined interstitial lung disease	14/19 (73.7%)
Current prednisone use*	3 (15.8%)
Current other immunosuppressant use†	4 (21.1%)
Current use of probiotic oral supplement‡	3 (15.8%)
Current use of proton-pump inhibitor	12 (63.2%)
Gastrointestinal tract 2.0 total score	Mean: 0.7 (0.6)§
Distension/bloating	Mean: 1.5 (0.9)§
Diarrhoea	Mean: 0.4 (0.6)**
Faecal soilage	Mean: 0.5 (0.9)**
Constipation	Mean: 0.7 (0.7)§
Emotional wellbeing	Mean: 0.5 (0.7)§
Social functioning	Mean: 0.5 (0.5)§

Values are n (%), except where otherwise noted.

* Dosages of prednisone was ≤10 mg daily.

† Immunosuppressant medications used included mycophenolate (n=2) and azathioprine (n=2).

‡ Probiotics used included Culturelle® (Amerifit, Inc., Cromwell, Connecticut, USA) (n=1), Florify® (Melaleuca, Idaho Falls, Idaho, USA) (n=1), and Align® (Procter & Gamble, Cincinnati, Ohio, USA) (n=1). Probiotics were not consumed for at least 3 weeks prior to the fecal sample collection.

§ Score indicates moderate symptom severity.[12]

** Score indicates mild symptom severity.[12]

ANA: antinuclear antibodies; HCRT: high-resolution computed tomography; IR: interquartile range; Scl-70: topoisomerase 1; SSc: systemic sclerosis.

The majority of patients had limited cutaneous disease (68.4%) and had ILD based on high-resolution CT of the chest (73.7%). Few patients consumed immunosuppressant medications during the study (Table 1).

None of the patients used tobacco products. Alcohol use was also reported infrequently; five patients reported that they had consumed alcohol within the month prior to the baseline stool collection. For the remaining stool collections, six reported alcohol consumption at Month 3, and three reported alcohol consumption at Months 6, 9, and 12, respectively. Alcohol consumption did not exceed three servings per week in any patient. Patients reported no changes in dietary patterns or restrictions during the study. Patients also reported no changes in their household (persons and pets living with them, or location of their home).

Longitudinal Stool Specimen Collection

The majority of participants (63.2%) provided at least four stool samples over the course of the 12-month study (provided five samples [n=6]; provided 4 samples [n=6]; provided three samples [n=2]; provided two samples [n=4]; provided one sample [n=1]). The patient who only provided one sample was not included in the longitudinal analysis. Two patients died during the study (both male) from respiratory failure caused by ILD. For the remaining participants, the reason for incomplete stool collection was forgetting to bring their sample.

Antibiotic Use During The Study

Six patients (31.6%) had taken antibiotics in the 3 months preceding the baseline stool sample collection; the mean time between cessation of antibiotics and stool collection was 6.5 weeks (range: 4.0–12 weeks). For the remaining sample collection time points, all patients stopped antibiotics at least 4 weeks prior to the sample collection with the exception of one patient who was taking ciprofloxacin at the time of the sample collection (3-month sample collection: one stopped 4 weeks prior, one stopped 6 weeks prior, one was on ciprofloxacin as mentioned above; 6-month sample collection: one stopped 8 weeks prior; 9-month sample collection: two stopped 4 weeks prior, one stopped 5 weeks prior; 12-month sample collection: one stopped 4 weeks prior). The indications for antibiotic use were predominantly infections (specifically digital ulcer infections), except for the one patient who received ciprofloxacin during the 3-month collection for the treatment of small intestine bacterial overgrowth. Only three patients reported consuming a commercial probiotic at baseline (Table 1), and all discontinued the probiotic at least 3 weeks prior to each stool collection.

Evolution of Gastrointestinal Tract Symptoms Over 1 Year

At baseline, the mean UCLA GIT 2.0 scores indicated moderate symptom severity[15] for the total score, as well as for the following specific domains: distension/bloating, social functioning, emotional wellbeing, and constipation (Table 1). The mean UCLA GIT 2.0 scores for faecal soilage and diarrhoea indicated mild symptom severity[15] (Table 1).

In the mixed effects model analysis, total UCLA GIT 2.0 score did not change significantly over the course of the 12-month study (p value for time trend: 0.555). Individual UCLA GIT 2.0 domain scores also did not change significantly over the course of the 12-month study, including the scores for constipation (p value for time trend: 0.617), distension/bloating (p value for time trend: 0.726), diarrhoea (p value for time trend: 0.115), social functioning (p value for time trend: 0.333), emotional wellbeing (p value for time trend: 0.898), and faecal soilage (p value for time trend: 0.345).

Disease Duration Affects the Course of Gastrointestinal Tract Symptoms

In univariable mixed effects model analysis, the only baseline characteristics associated with the course of the total GIT score and the GIT scores for the individual domains were disease duration and SSc subtype (limited versus diffuse disease). When disease duration and SSc subtype were included as covariates in the models, only disease duration remained associated with the outcome (Table 2). Specifically, patients with longer disease durations had increased symptoms over time for the following: total GIT score, bloating, faecal soilage, diarrhoea (trend), social functioning, emotional wellbeing, and constipation.

Table 2: Baseline characteristics associated with the course of total systemic sclerosis-gastrointestinal tract symptoms based on mixed effects model analysis.

Variable	Estimate	Standard error	p value
SSc subtype (limited)	-0.138	0.219	0.532
Disease duration (years)	0.0380	0.0130	0.0038
Time (continuous)	0.0080	0.0070	0.2760

SSc: systemic sclerosis.

Longitudinal Assessement of the Gastrointestinal Tract Microbiome

Microbial composition remained stable within subjects over the course of 1 year. Specifically, there was no significant difference in alpha diversity over the course of the study as measured by Chao1 index (p value: 0.78) and by the Shannon index (p value: 0.76). There was also no significant association between UCLA GIT 2.0 score longitudinally with the Chao1 and Shannon indices (p value: 0.44 and 0.27, respectively). The beta diversity analysis also showed no significant within-subject changes over the course of the study (p value: 0.68) and no significant association with UCLA GIT 2.0 score longitudinally.

Moreover, mixed effects models generated for each individual taxon at the genus level demonstrated that the relative abudance of each genus did not change significantly over time (all q values: >0.1). Moreover, if a specific genus was present or absent in a given subject at baseline, it remained present or absent at all of the subsequent analysis time points throughout the year-long study. Thus, this study found no change in individual taxonomic abundances at the genus level within each subject over 1 year.

Specific Genera are Associated with Gastrointestinal Tract Symptoms Over Time

Because GIT score and the abundance of specific genera did not change significantly with time, GIT scores and genus level abundances were averaged across all time points for every subject and entered into a rank regression analysis. The results of this analysis revealed that specific genera were associated with the course of GIT symptoms over 12-months, even after controlling for age, sex, ethnicity, SSc type (i.e., limited versus diffuse), and SSc disease duration. For example, increased abundance of *Bacteroides*, *Prevotella*, and specific genera from the Clostridiales order were associated with improvement in the course of total GIT symptoms over 1 year (Table 3).

DISCUSSION

This is the first study to longitudinally characterise the GIT microbiota of patients with SSc. The findings suggest relative stability of the GIT microbiome in SSc patients over the course of 1 year, with no appreciable changes in alpha and beta diversity or the relative abundance or presence/absence of specific genera. The study also demonstrated that self-reported GIT symptoms also do not change significantly over the course of 1 year in patients with SSc. However, consistent with prior studies,[5,6] specific genera were associated with GIT symptoms.

The observation that GIT symptoms did not change significantly over the course of 1 year within subjects was initially suprising because many patients with SSc experience day-to-day variations in the severity of their GIT symptoms. However, since the GIT 2.0[20] asks patients to recall symptoms over the course of 1 week, it is plausible that these day-to-day variations were not captured. Since few studies have evaluated longitudinal progression of GIT symptoms, it is also possible that progression occurs at a relatively slow rate in patients with SSc. A study of longer duration (>1 year) may be needed to accurately estimate the rate of progression of GIT symptoms in patients with SSc.

Table 3: Summary of microbial genera associated with decreased gastrointestinal tract symptoms over 1 year in patients with systemic sclerosis.

Genera	GIT domain	Estimate	Standard error	q value
Bacteroides	Total GIT	-0.234	0.089	0.023
*Finegoldia**	Distension/bloating	-0.474	0.267	0.103
*Peptoniphilus**	Distension/bloating	-0.424	0.229	0.091
*Pseudomonas**	Distension/bloating	-0.201	0.084	0.036
Alloscardovia†	Diarrhoea	-0.389	0.140	0.014
Paraprevotella‡	Diarrhoea	-0.128	0.051	0.024
Prevotella‡	Diarrhoea	-0.069	0.030	0.039
Undefined genera*	Diarrhoea	-0.186	0.070	0.019

* Genera from the Clostridiales order.
† Genera from the Bifidobacteriales order.
‡ Genera from the Bacteroidales order.
GIT: gastrointestinal tract.

The only variable found to affect the progression of SSc-GIT symptoms in this cohort was disease duration. Specifically, patients with longer disease duration had increased GIT symptoms over time. SSc subtype was not associated with the course of GIT symptoms when disease duration was considered. In line with these findings, Lock et al.[22] reported that the prevalence of lower GIT symptoms were similar between patients with diffuse and limited cutaneous disease. The results of this study suggest that GIT symptoms should be carefully monitored in all patients, regardless of their SSc cutaneous subtype; however, patients with longer disease duration may require more vigilant monitoring.

Another unexpected finding of the present study was that the presence/absence of specific genera, as well as the relative abundances of specific genera, did not change significantly over the course of the study. These findings may be because patients consumed a relatively stable Western diet throughout the study period. While seasonal microbiota variations have been observed in some populations, such as the Hadza in Tanzania,[23] such variations would not be expected among most individuals living in the USA. None of the patients reported significant changes in dietary patterns (e.g., adopting a gluten-free diet or plant-based diet for instance) that have been found in prior to studies to influence GIT microbiota.[24,25]

Consistent with prior studies using colonic lavage specimens[5] and faecal specimens,[6] certain genera were associated with the course of the GIT symptoms. Specifically, increased abundance of *Bacteroides* was associated with decreased total GIT score over time. This finding is consistent with a prior study, which demonstrated that patients with higher *B. fragilis* in both the cecum and sigmoid regions of the colon had decreased bloating/distension, diarrhoea, and total GIT symptoms.[5] Considered a commensal genus by several host inflammatory and physiologic endpoints in animal models, *Bacteroides* was significantly lower in SSc patients from two geographically distinct cohorts (e.g., Oslo Univeristy Hospital [OUH], Oslo, Norway; and UCLA, Los Angeles, California, USA) compared to healthy controls.[6] The fold change scores for both the UCLA-SSc and OUH-SSc cohorts relative to controls was nearly five, signifying a substantial shift in this genus in SSc.[6] Low relative abundance of this genera is associated with increased disease activity in other autoimmune diseases, including Crohn's disease.[26] The present findings suggest that through increasing the abundance of *Bacteroides*, patients with SSc may potentially experience an improvement in GIT symptoms, and future interventional studies are needed to test this hypothesis.

In addition to *Bacteroides*, specific genera from the Clostridiales order (e.g., *Finegoldia*, *Peptoniphilus*, and *Pseudomonas)* were associated with decreased distension/bloating (see Table 3 for further details). In prior studies,[5,6] other genera from the Clostridiales order (namely, *Clostridium* were associated with decreased total GIT symptom severity score, as well as GIT scores for constipation and the bloating/distension.[5,6] In addition, *Clostridiaceae* was decreased among SSc patients with dysbiosis in a Swedish cohort study compared with eubiotic patients.[7] Future therapeutic efforts may also consider increasing the abudance and/or activity of genera from the Clostridales order.

The findings of the present study should be considered within the context of certain limitations. First, the sample size is small, and the study may not be adequately powered to detect significant changes in symptoms/microbial composition over time. However, despite the small sample size, the significant associations between genera and symptoms were consistent with associations reported in prior studies,[5,6] suggesting that the present findings are unlikely to be caused by chance alone. Second, to evaluate changes in specific genera over time, numerous inference tests were performed, which may have inflated the Type 1 error rate. To mitigate the risk of Type 1 error, the study used a relatively conservative FDR to correct for multiple hypothesis tesing. In addition, this study did not include a validation cohort.

Furthermore, this study did not include an objective assessment of GIT transit. Andreasson et al.[7] found that the degree of dysbiosis correlated with the degree of dysmotility (based on cineradiography of oesophageal function). No studies have evaluated the effects of lower GIT dysmotility on microbiota in SSc. It is unclear whether dysmotility or the GIT pathology of SSc itself drives changes in microbial composition, or if it is the changes in microbial composition influencing GIT motility in SSc. To answer this question, longitudinal studies are needed, which will evaluate changes in microbial composition in conjunction with changes in motility over time.

In addition, it may be prudent to systematically assess dietary intake patterns in future SSc-GIT microbiome studies. In a relatively large study (N=1135), Zhernakova et al.[27] identified 60 dietary factors affecting variations observed in the inter-individual distance of microbial composition. Assessing dietary intake is challenging because of recall bias; however, dietary modifications represent an important and low-risk means of altering GIT microbial composition and metabolic output.[28,29] For instance, adoption of a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) led to signficant alterations in the microbiome metabolome of patients with irritable bowel syndrome.[30]

The present study also has important strengths. By studying microbial composition at multiple time points, the likelihood that the observed associations are caused by chance alone has been minimised. In addition, by performing all of the sequencing analyses simultaneously, the possibility of a batch effect has been eliminated. The authors also took caution to ensure that all patients withheld medications, such as antibiotics and probiotics, at least 3 weeks prior to the stool collection, by verifying the medication lists on three occasions in the month preceding the collection.

FUTURE DIRECTIONS

While the present study did not detect changes in the microbial composition over the course of 1 year, longer studies may be needed to assess if and how the GIT microbiome evolves over time in this disease state, particularly from the time of the initial diagnosis to the development of specific manifestations of SSc, such as ILD. Such studies may uncover novel microbial predictors of organ involvement in SSc. In addition, larger studies are necessary, which are adequately powered to detect important microbiota associations with specific SSc disease features.

CONCLUSION

This study demonstrated that both GIT symptoms and dysbiotic microbial composition remain relatively stable over the course of 1 year in patients with SSc. This study also identified specific bacterial genera associated with the SSc-GIT symptoms and was the first to do so in the

context of a longitudinal study. Additional studies are needed to validate and expand upon these findings, particularly among patients with early SSc. Future studies may also assess whether manipulation of the GIT microbiome can lead to symptomatic improvement of SSc-GIT symptoms.

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Prostate Cancer Screening Recommendations for General and Specific Populations in the Western Nations

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Disclosure: The authors have declared no conflicts of interest.

Received: 24.02.20

Accepted: 21.05.20

Keywords: Individualised medicine, metastasis, prostate cancer (PCa), prostate-specific antigen (PSA), screening, United States Preventive Services Task Force (USPSTF).

Citation: EMJ. 2020;DOI/10.33590/emj/20-00042.

Abstract

There is a chaotic scenario that exists in the field of prostate cancer (PCa) screening. To balance goals, such as decreasing mortality, avoiding unnecessary procedures, and decreasing the cost of medical care, the pendulum seems to have swung to the side of more restricted screening. The decrease in PCa screening has led to a slowly creeping decline in the favourable outcomes that existed among patients with PCa. If a potential patient or a family member is trying to get clear guidance about PCa screening by searching the internet, they will end up confused by several recommendations from many organisations. It is even more challenging to obtain any clarity about PCa screening for special populations, such as those with a family history of PCa, those of African descent/African Americans, and the elderly. The advent of genomic medicine and precision medicine is an opportunity to identify those at a very high risk of developing aggressive PCa, so that PCa screening can be more actively undertaken among them. In this paper, the authors review the current recommendations by different entities and summarise emerging molecular markers that may help bring clarity to PCa screening. The authors predict that concrete, consensual guidelines will emerge in less than one decade. Meanwhile, this article suggests intermediary steps that will help save lives from PCa mortality, especially for under-represented populations. This paper is a catalyst to stimulate further discussion and serves as a guide to noncancer-specialists for the near future as precision medicine progresses to better understand risk–benefit and cost–benefit ratios in PCa screening.

INTRODUCTION

Prostate cancer (PCa) was the most commonly diagnosed malignancy in 105 countries in 2018 and the fifth leading cause of cancer deaths in males.[1] In general, approximately one in nine males will be diagnosed with PCa during their lifetime.[2] Despite the high incidence of PCa in the USA and worldwide, PCa is a very indolent disease. In the USA, an average of 2.44% of males will die from PCa.[3] In a study by Johansson et al.,[4] 223 untreated patients with PCa were followed for >30 years and showed 41% local progression, 18% progression to distant metastasis, and a mortality rate of 18%. The mean time to development of metastasis was 9.2 years and the mean time to death was 9.5 years.[4] The outcome of PCa is highly dependent on the grade classification at diagnosis. A study by Albertsen et al.,[5] which followed a cohort of 767 untreated patients with PCa for 15 years, found a 4–11% mortality rate for Gleason Score 2–5, 18–30% for Gleason Score 6, 42–70% for Gleason Score 7, and 60–87% for Gleason Score 8–10.[5]

The introduction of prostate-specific antigen (PSA)-based screening had a large impact on PCa. Following the introduction of PSA screening, the mean age at diagnosis of PCa decreased.[6] The incidence of males with nonorgan-confined PCa decreased from 79.3% in 1984 to 24.7% in 2005.[7] Most significantly, the 5-year survival rates for all races improved from 68% in 1975–1977 to 100% for the years 2003–2009.[8] Although PSA screening appeared highly beneficial, these results were all retrospective and raised the question of whether PSA screening was actually improving survival or simply detecting earlier and possibly insignificant PCa and in doing so, causing potential harm. Results of prospective studies led to the first recommendations regarding PSA-based PCa screening, which had a large and controversial impact on PCa. While the potential benefit of PCa screening is evident, the question of who and when to screen remains. The authors hypothesised that the concept of individualised medicine can be applied to PCa screening to optimise its benefit to males most at risk.

METHODOLOGY FOR ARTICLE SEARCH CRITERIA

To identify the available and recommended PCa screening tests, the authors completed a PubMed search using keywords: "prostate cancer", "prostate cancer biomarkers", "prostate cancer and race", "MRI in prostate cancer", and "prostate cancer screening guidelines". Only the studies reported in the English language were included. The authors selected the studies published within the past 10 years, as well as reported the secondary sources from reference lists of retrieved articles online.

2012 U.S. PREVENTIVE SERVICES TASK FORCE SCREENING GUIDELINES AND THEIR IMPACT

Table 1 summarises various agencies and their associated PCa screening guidelines. In 2012, the U.S. Preventative Services Task Force (USPSTF) released a recommendation against the use of PSA-based screening for PCa in all males (Grade D).[9] Previously, the USPSTF had recommended against PCa screening in males over the age of 75 years (Grade D), but had found data insufficient to assess males younger than 75 years old (Grade I).[10] The 2012 recommendation was largely based on the results of two trials: the U.S. Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial and the European Randomized Study of Screening for Prostate Cancer (ERSPC).[9]

The PLCO trial was conducted at 10 centres in the USA.[11] The study randomised 76,693 males aged 55–74 years to annual screening or usual care. Initial results of a 7–10-year follow-up showed no difference in PCa mortality between the two groups (rate ratio: 1.13; 95% confidence interval [CI]: 0.75–1.7). Follow-up at 13 years also failed to show any significant difference in mortality rates (rate ratio: 1.09; 95% CI: 0.87–1.36).[12]

The ERSPC was a multicentre, population-based randomised screening trial conducted in eight European countries.[13] A total of 162,389 males aged 55–69 years were randomised to a group that offered PSA screening every 4 years or a control group that did not offer screening.

Table 1: Selected international prostate cancer screening guidelines.

Agency	Screening guideline	Screening frequency	Biopsy guideline
AAFP	Shared decision-making aged 55–69 years. No screening aged >70 years.	Every 2 years at most.	PSA ≥4 ng/mL
ACP	Shared decision-making aged 50–69 years. No screening aged <50 years, >69 years, or <10-year life expectancy.	No clear benefit for more frequency than every 4 years. PSA >2.5 ng/mL may be warranted yearly.	N/R
ACPM	No routine screening. May be considered for high-risk patients.	N/R	N/R
ACS	Shared decision-making if >10-year life expectancy. Starting aged 50 years for males at average risk and younger for those at high risk.	Yearly if PSA >2.5 ng/mL, otherwise every 2 years.	PSA ≥4 ng/mL or PSA >2.5 ng/mL and risk factors.
AGS	Do not recommend without considering life expectancy and risk.	N/R	N/R
AMDA	No screening if <10-year life expectancy.	N/A	N/R
ASCO	Shared decision-making if >10-year life expectancy. No screening if 10-year life expectancy and no symptoms of prostate cancer.	N/R	N/R
AUA	Shared decision-making aged 55–69 years. None for those aged <40 years. No routine screening if aged 40–54 years, >70 years, or <10–15-year life expectancy (may consider for high risk).	1–2 years.	Use predictive tools to better qualify risk of prostate cancer.
CCO	Shared decision-making.	N/A	N/R
CTFPHC	Recommend against screening for prostate cancer.	N/A	N/R
CUA	Shared decision-making males aged 50–70 years (60 years if PSA <1 ng/mL). Offer at age 45 years if high risk. No screening if life expectancy <10 years.	Every 4 years in PSA <1 ng/mL. Every 2 years of PSA 1–3 ng/mL. More frequent or additional testing if PSA >3 ng/mL.	Shared decision-making. For PSA >3 ng/mL consider additional strategies.
EAU–ESTRO–SIOG	Individual risk-adaptive strategy. Inform of risk and benefits. Males aged >50 years, aged >45 years with family history, or African American males aged >45 years. Only screen if good performance status and at least 10–15-year life expectancy.	Every 2 years if initial PSA >1 mg/mL at age 40 years or if PSA >2 ng/mL at age 60 years. Otherwise screen every 8 years.	Abnormal DRE or PSA >10 ng/mL. If normal DRE and PSA 2–10 ng/mL, obtain risk calculation, imaging, or further testing.

Table 1 continued.

Agency	Screening guideline	Screening frequency	Biopsy guideline
ESMO	Population-based screening is not recommended. Testing for prostate cancer should not be done in asymptomatic males aged >70 years.	N/R	Only after repeat PSA. Consider age, PSA, DRE, comorbidities, free/total PSA, previous biopsy, and patient values.
NCCN	Shared decision-making aged 45–75 years (40 years if high risk). Screen age >75 years only if good health.	PSA <1 ng/mL: 2–4 years. PSA 1–3ng/mL: 1–2 years.	Aged 45–75 years with PSA >3 ng/mL or very suspicious DRE. Aged >75 years with PSA >4 ng/mL or very suspicious DRE.
NCI	Inadequate evidence to recommend screening.	N/A	N/R
NHS	Shared decision-making if requested starting at age 50.	N/R	N/R
RCP	Shared decision-making.	N/A	N/R
USPSTF	Shared decision-making age 55–69. No screening age >70.	N/R	N/R

AAFP: American Academy of Family Physicians; ACP: American College of Physicians; ACPM: American College of Preventative Medicine; ACS: American Cancer Society; AGS: American Geriatrics Society; AMDA: The Society for Post-Acute and Long-Term Care Medicine; ASCO: American Society of Clinical Oncology; AUA: American Urological Association; CCO: Cancer Care Ontario; CTFPHC: Canadian Task Force on Preventative Health Care; CUA: Canadian Urological Association; DRE: digital rectal examination EAU: European Association of Urology; ESMO: European Society for Medical Oncology; ESTRO: European Society for Radiotherapy and Oncology; N/A: guideline not available; N/R: not recommended; NCCN: National Comprehensive Cancer Network; NCI: National Cancer Institute; NHS: National Health Service; PSA: Prostate Specific Antigen; RCP: Royal College of Pathologists; SIOG: International Society of Geriatric Oncology; USPSTF: United States Preventative Services Task Force.

At the time of the 2012 USPSTF recommendations, the ERSPC showed a significant reduction in the risk of PCa mortality (approximately 20%); however, nearly 1,000 males needed to be screened to prevent one death.

Based on the results of the PLCO and ERSPC studies, the USPSTF concluded that the potential benefits of PSA-based screening were "at best, very small."[9] The USPSTF also evaluated the risks and harms associated with screening. They considered the high rate of false-positive results, complications of biopsy, and side effects of treatment. These negative consequences of screening were compounded given the perceived high rate of overdiagnosis because of the often-indolent nature of PCa. Thus, the USPSTF concluded with moderate certainty that the benefits of PCa screening did not outweigh the harm, leading to the 2012 recommendations, as mentioned above, against PSA-based screening. The recommendation led to much controversy and numerous studies evaluating the impact on PCa.

The first and most direct impact of the 2012 USPSTF recommendation was on PCa screening rates. Studies using various metrics have shown a decrease in PSA screening rates after 2012.[14-16] PCa screening is largely carried out by primary care providers (PCP) and urologists. A 2016 study by Zavaski et al.[16] used the National Ambulatory Medical Care Survey (NAMCS) and compared the rates of PSA screening between 2010 and 2012 for PCP and urologists.[16] PCP saw a decrease in PSA screen rates from 36.4% to 16.4% (adjusted odds ratio: 0.44; 95% CI: 0.24–0.80). Urologists, who rely less heavily on the USPSTF, saw a decrease in PSA screening rates from 38.7% to 34.5% (adjusted odds ratio: 0.89; 95% CI: 0.19–1.84). Two large population studies showed a

significant decrease in PSA screening after 2012. A 2015 study by Drazer et al.[17] looked at data from the National Health Interview Survey (NHIS) and showed a decline in PSA screening between 2010 and 2013.[17] Rates declined for males aged 50–59 years (33.2% to 24.8%, p<0.01), aged 60-74 years (51.2% to 43.6%, p<0.01), and aged ≥75 years (43.9% to 37.1%, p=0.03).

This significant nationwide decrease was also seen in a 2016 study by Jemal et al.[15] using Surveillance, Epidemiology, and End Results (SEER) data, which showed an 18% decrease in screening rates between 2010 and 2013. The decrease in PCa was greatest among males aged 55–74 years.

Consistent with a decrease in PCa screening rates following the 2012 USPSTF recommendations, studies have shown a decrease in both prostate biopsy rates and incidence of PCa.[18-21] A 2017 study by Halpern et al.[21] evaluating the number of procedures performed by urologists in the USA from 2009 to 2016 found that the number of prostate biopsies decreased by 28.7% after 2012 (parameter estimate: -0.25; standard error: 0.03; p<0.001).[21] Barocas et al.[18] analysed the National Cancer Database (NCDB) comparing PCa incidence in 2010 and 2012, and found a 28% decrease in the incident diagnosis of PCa the year after the release of the USPSTF draft.[18] A 2019 analysis by Butler et al.[19] of the SEER database comparing PCa incidence rates between 2010 and 2015 showed a decrease in the incidence (per 100,000 people) of localised prostate from 195.4 to 131.9 (p<0.001) among males aged 50–74 years and from 189.0 to 123.4 (p<0.001) among males aged ≥75 years.[19] However, the decline in PCa incidence has not necessarily been uniform over all risk groups.

A 2019 analysis of the NCD by Fletcher et al.,[20] looking at males with clinically localised PCa (T1–4, N0, M0) from 2004 to 2014, showed a shift towards higher-risk PCa.[18] They found that among 755,567 males diagnosed with PCa, low-risk PCa decreased (38.32% to 27.23%, p<0.001), while increases were seen in intermediate-risk (40.49% to 46.70%, p<0.001) and high-risk (21.19% to 26.05%, p<0.001). The increase in intermediate and high-risk PCa were largely because of an increase in Gleason Score. Several studies have shown an increase in Gleason Score and a general higher-risk, more aggressive PCa following the 2012 recommendations.[19,20,22]

Most concerning, numerous studies have documented an increase in the rate of metastatic PCa.[19,20,22,23] The 2019 SEER analysis by Butler et al.[19] showed an increase in the incidence of metastatic PCa from 6.2 to 7.1 (p<0.001).[19] Using SEER data, Kelly et al.[23] further analysed the change in metastatic PCa.[23] The authors found that metastatic PCa declined by 1.45% per year from 2004 to 2007 and then increased by 0.58% per year after 2008 and 2.40% per year after 2012. They had forecasted that metastatic PCa would continue to increase by 1.03% per year through to 2025, leading to an increase in the annual burden by 42%. It remains to be seen if the increasing aggressiveness, risk group, and rate of metastatic disease translates into a decrease in survival.

CRITICISM OF 2012 USPSTF RECOMMENDATIONS AND 2018 UPDATE

Several criticisms exist regarding the studies used to guide the 2012 USPSTF recommendation against PSA-based PCa screening. The PLCO trial has been considered highly flawed and not a true comparison of screening versus not screening, as a high number of males were screened regardless of randomisation group and a low number of males in screening groups received biopsies. Specifically, 44% of all those enrolled had at least one PSA screen before enrolment, 40–52% in the control group were offered PSA screening per year (resulting in 79% having had at least one PSA screen at some point in the trial), and only 41% of males in the screening arm received biopsy following a positive PSA result.[11,24] A chief concern in the use of the ERSPC results is that, at the time of the 2012 USPSTF analysis, only 9 years of follow-up had been reported.[13] Given that PCa is a highly indolent disease and factoring in 11–12 years of lead time bias because of screening, 9 years may have been too short an interval to truly gauge the impact of PSA screening.[4,25] Subsequent follow-ups of the ERSPC trial have shown continued improvement with time.[26,27]

Following the 2012 recommendations, the USPSTF conducted a review of studies published between July 2011 and February 2018.[28] Sixty-three studies in 104 publications were included in their analysis. Of the studies evaluated, three were randomised controlled trials: the PLCO trial, the ESRCP trial (13-year update), and

the Cluster randomised trial of PSA testing for prostate cancer (CAP). These three trials largely constituted the studies used to gauge the impact of PSA screening. The PLCO trial and the CAP trial showed no benefit to PSA screening. The ESRCP update showed a reduction in the incidence of metastatic PCa (relative risk: 0.7; 95% CI: 0.6–0.82) and a reduction in the number needed to screen to prevent one death from PCa to 781.

Based on the continued literature review, the USPSTF released updated PCa screening recommendations in 2018.[29] They continued to recommend against PSA-based screening for males aged ≥70 years (Grade D). However, for males aged 55–69 years, they advised PSA-based screening based on a shared decision-making approach between physician and patient. The USPSTF continued to note the potential harms of PSA screening (false positives, side effects of biopsy, over diagnosis, and side effects of treatment). The improvement in prevention of PCa deaths and in development of metastatic disease seen in the ESRCP trial now warranted consideration of screening. Specifically, that the decision to screen should be an individual one after being informed of the risk and the benefits of screening (Grade C).

The 2018 updates also reviewed and discussed the role of screening in African American males and those with family history of PCa.[29] They noted that the two groups, particularly African American males, had an increased risk of PCa that might further increase the benefits of PSA-based PCa screening. Unfortunately, existing data were insufficient to fully evaluate PSA-based screening in these specific subgroups. The ESRCP trial did not record data for race but given the comparatively low percentage of those of African descent in Europe, this likely means that the group was not well represented.

The ERSPC trial continues to show increasing benefit to PSA-based PCa screening. In 2019, a 16-year update was published, which showed a further reduction in the number needed to screen to prevent one PCa death to 570.[30] This is within numbers needed to screen that are observed with other recommended cancer screening tests, including those for breast cancer and colon cancer. It should be noted that although the median follow-up time in the ESRCP trial was 15.5 years from randomisation, the median follow-up time from PCa diagnosis was only 8.8 years in the screening group and 5.4 years in the control group.[29] Future updates may likely continue to show increasing benefit to PCa screening. Given their greater risk of aggressive PCa, the benefits seen in the ESRCP trial may indicate an even greater potential benefit in African Americans.

OTHER AGENCY GUIDELINES: AMERICAN UROLOGICAL ASSOCIATION AND EUROPEAN ASSOCIATION OF UROLOGY RECOMMENDATIONS

The USPSTF recommendations are influential, but other agencies also issue PCa screening guidelines. Following the 2012 USPSTF recommendation, the American Urological Association (AUA) released guidelines in 2013, which were reviewed and reaffirmed in 2018, recommending that males aged 55–69 years be offered PSA screening based on shared decision-making. Furthermore, they recommended against routine screening in males aged 40–54 years, but noted it may be considered for high-risk patients.[31] The European Association of Urology (EAU) has issued similar guidelines recommending a risk-adaptive, shared decision-making approach for average-risk males aged >50 years and high-risk males aged >45 years.[32] Overall, many agencies are transitioning to an individualised, shared decision-making approach. Table 1 summarises various agencies and their associated PCa screening guidelines.

PROSTATE CANCER SCREENING FOR AFRICAN AMERICANS OR MALES OF AFRICAN DESCENT

African American males are more likely to develop PCa, develop it at a younger age, and are twice as likely to die from PCa.[29] These differences can be attributed to differences in the genetic make-up of the cancer,[33] reduced access to screening[34] or inadequate follow-up after screening,[35] or difference in treatment.[36] The USPSTF bases its recommendation on three randomised controlled trials in which the African American males were under-represented, for example, with 4% in the PLCO trial.[16,29,37] These trials were not enough to determine if there any different screenings needed for African American males.[29] Potential risk of harm was higher for African American males compared with that for Caucasian males,

reported an analysis of the PLCO trial.[38] Given the higher incidence and mortality among African American males, this population is more likely to benefit from PSA screening than the general population, but further studies are warranted to confirm this hypothesis. The USPSTF currently does not recommend more aggressive screening for African American males because of insufficient evidence; however, they believe that a reasonable approach would be for physicians to inform their patients who are African American about their increased risk and potential benefits and harms of screening, thus helping to make an informed, personalised decision about PCa screening.

AFRICAN AMERICAN-SPECIFIC PROSTATE CANCER SCREENING GUIDELINES: A SURVEY OF SPECIALISTS

PCa in African American males has several unique epidemiologic, genetic, and clinical features compared to PCa in Caucasian males, so it may have been inappropriate to apply the 2012 USPSTF PCa screening guidelines within the African American population. The authors had previously hypothesised that the higher mortality associated with PCa among African American males might be reduced by designing a race-specific screening schema. The authors then conducted a survey that would poll a panel of PCa specialists about various options available to develop a unique screening guideline for African American males. Their responses and opinions were used to propose a novel PCa screening guideline for African American males.

The majority of surveyed PCa specialists believed African American males should be screened using distinct PCa screening. Using input from the expert panel, the authors presented a unique screening protocol for African American males. The presurvey schematic, shown in Figure 1, was modified using the results of the survey to produce a schema, which more closely aligns to the views of the expert panel, shown in Figure 2.

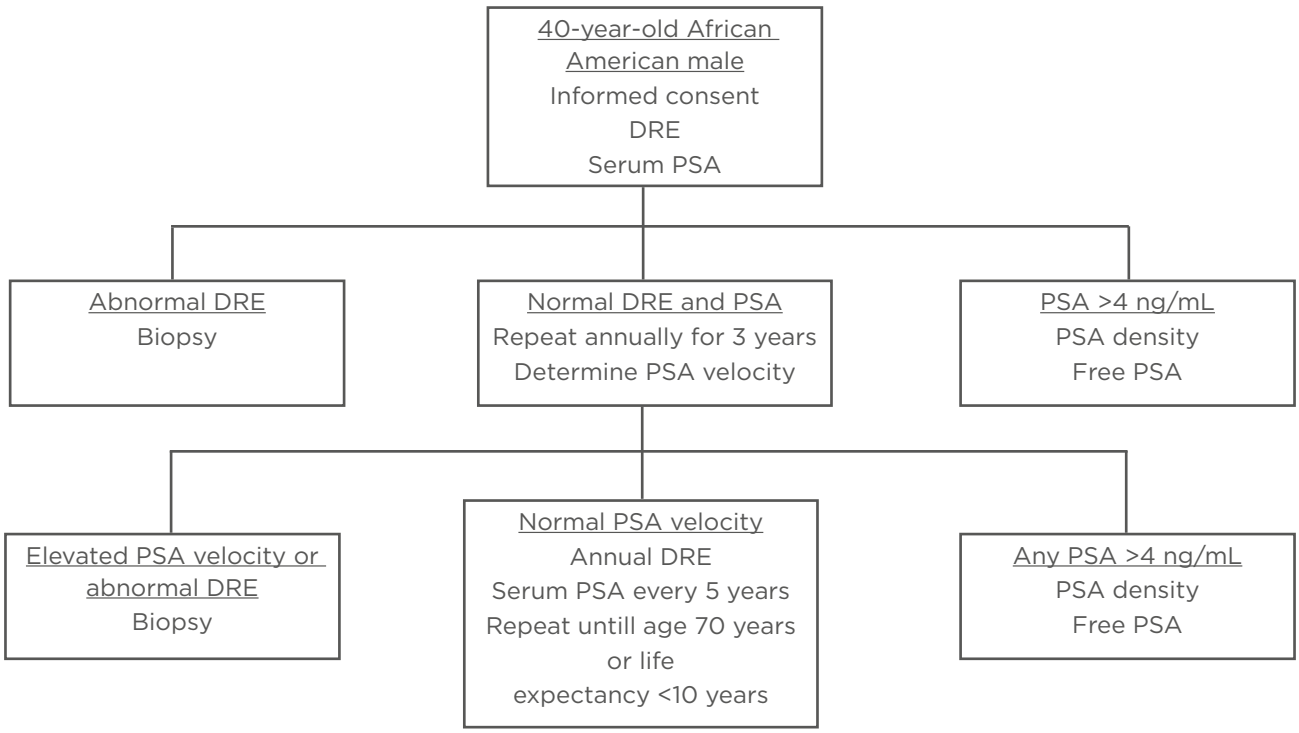

Figure 1: Screening schematic for African American males proposed prior to interviewing experts.

DRE: digital rectal examination; PSA: prostate specific antigen.

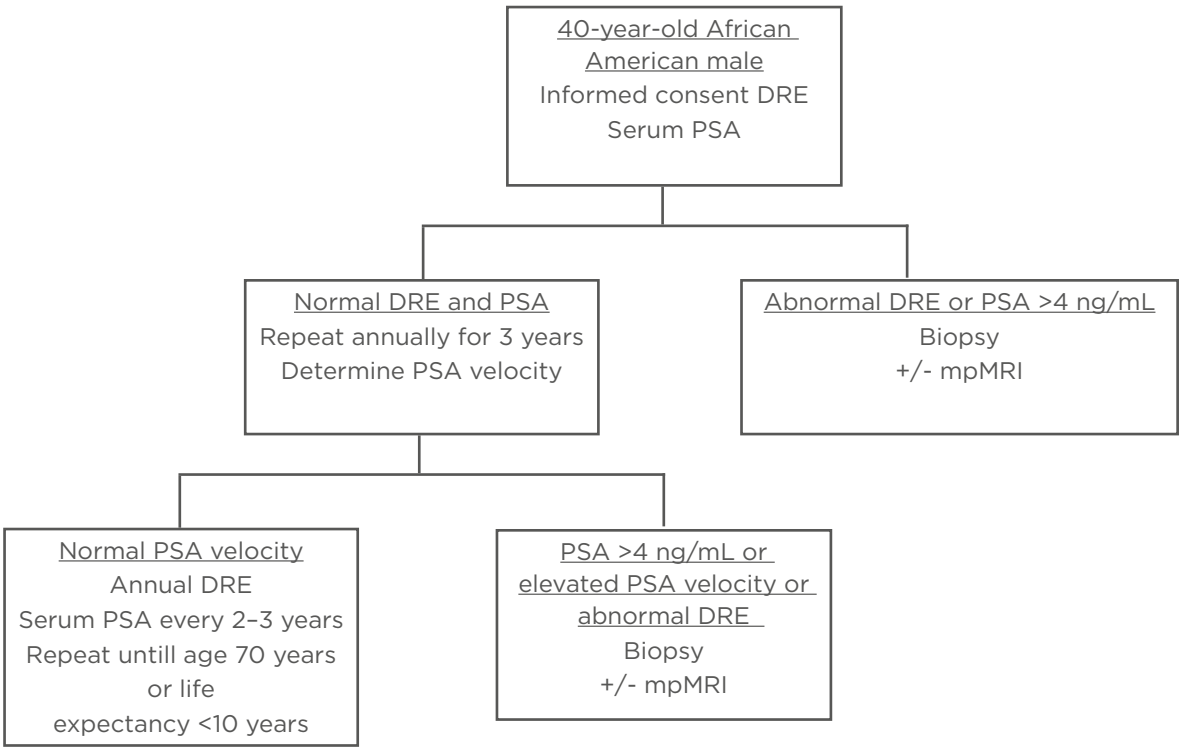

Figure 2: Revised screening schematic for African American males after interviewing experts.

PSA: Prostate Specific Antigen; DRE: digital rectal examination; mpMRI: multiparametric MRI.

Three notable modifications were made: at the initial screening (at 40 years of age) in the setting of a serum PSA >4 ng/mL, patients would proceed directly to biopsy rather than PSA density or free PSA; during annual digital rectal examination and PSA tests, if any PSA is reported as >4 ng/mL, patients are to proceed directly to biopsy; thirdly, in the setting of normal PSA velocity, serum PSA should be measured every 2–3 years, rather than at 5-year intervals.

This study was limited by a low response rate and had potential for confounding bias. Physicians who stand to benefit (financially, for example) from additional screening have an inherent conflict of interest.

A multivariate analysis should have been performed to control for this bias and provide additional information about the opinions expressed in the survey (Vijayakumar, personal communication).

The majority of surveyed PCa specialists believed that separate PCa screening guidelines for African American males should exist because of their unique epidemiological, genetic, social, and clinical features. The authors constructed and presented a novel screening protocol for African American males using the input from the expert panel. Considering the USPSTF release of updated PCa screening guidelines that encourage physicians and patients to make an informed discussion about PCa screening, the timing is appropriate to introduce specific screening guidelines for African American males.

THE ROLE OF MRI IN PROSTATE CANCER SCREENING

PCa is a common, serious disease for which outcomes improve if caught earlier in its course. Unfortunately, the task of screening is complicated by the large prevalence of low-grade disease. The history of PCa screening has been marred by test results that have led to unnecessary fears and procedures in patients whose disease would likely have never become problematic.[9] New and improved screening guidelines will need to look to new modalities to rightly discern between indolent and sinister disease, and MRI may be able to play a vital

role if its current limitations can be overcome. Unfortunately, much of the data on the ability of MRI to screen for PCa is extrapolated from patients known to have elevated PSA levels, but that will soon to change.[39] There is an ongoing randomised controlled trial comparing multiparametric-MRI-aided screening guidelines to those using PSA, which will achieve results in June 2020. The potential advantages of MRI-aided screening guidelines are three-fold. First, from available data, multiparametric-MRI appears to have a high negative predictive value in populations of low cancer incidence, which would be maximised in a screening setting.[40] Secondly, there is potential for MRI to preferentially detect high-grade disease as it has been shown to be less sensitive in low-grade disease.[41] Thirdly, prospective data have shown that biopsy of a lesion detected by MRI has a much higher negative predictive value than standard transrectal ultrasound-guided biopsy.[42] There seem to be two dominant limitations of using MRI in a screening schema: inter-reader variability and cost. A study by Branger[43] showed that a negative MRI could not reliably exclude Gleason pattern 4 disease or extracapsular extension, but in the hands of an experienced radiologist, disease of core length ≥5 mm or Gleason Score ≥7 negative predictive values exceeded 95%. On an individual level, the cost of a prostate MRI is similar to the cost of a screening colonoscopy.[44] However, there may be an overall cost advantage when considering the potential system-wide reduction in unnecessary biopsies and treatments. In the event that multiparametric-MRI is shown to be superior to PSA-based guidelines, economic studies will be required. In conclusion, MRI-based PCa screening has potential to offer a more appropriate screening measure than current methods, but its utility and cost-effectiveness must be demonstrated first.

COST–BENEFIT ANALYSIS

Several different strategies have been utilised to determine cost-effectiveness of PSA screening. These include, but are not limited to, no screening, biennial screening from age 40–74 years, a single screening at age 60 years, screening every 4 years from age 55–69 years, and screening every 4 years from age 50–74 years. In a Canadian study, Pataky et al.[45] studied the cost-effectiveness of PSA screening using an existing model of PCa. They determined that PCa mortality reductions occurred with 4-year PSA screening of 55–69-year-old males, as well as with 2-year screenings of those aged 40–74 years. However, this model also projected an increase in overdiagnosis with either screening strategy.[45]

In a microsimulation model, it was found that out of a population of 1,000 males in a nonscreening scenario, there were 136 cases of PCa diagnosis, 35 deaths, and 246 negative biopsies. However, in the population for the screening scenario of 1,000 males, the model showed 178 diagnoses of PCa and 27 deaths, with 443 negative biopsies. In this model, it was predicted that PSA screening every 4 years increased the cost for PCa by 44%, with eight fewer deaths per 1,000 males.[46] Although the cost of a PSA screening is $30–100 USD, the vast majority of the cost of PCa can be accredited to diagnosis and treatment. The rate of false positive PSA screenings leads to an increase in unnecessary diagnostic biopsies and the associated risk of undergoing these procedures.[47] There is a need for better PCa screening that will reduce the number of false positives, increase the number of true positives, and greatly reduce the cost of diagnosing and treating PCa.

FAMILIAL HISTORY AND THE RISK OF PROSTATE CANCER INCIDENCE AND SEVERITY

Factors that have been identified as familial risks for a PCa include being a male of African American descent, having a first-degree relative with a history of PCa, and advancing age. Using the Swedish Family-Cancer Database, it was found that males who had a father, brothers, or both, with a history of PCa were at an increased incidence of developing the disease.[48] Further, Barber et al.[49] determined that males with a family history of either breast cancer or PCa had an increased risk of PCa.[49] Family history of PCa alone was associated with a 68% increased risk of total disease and a 72% increased risk of lethal disease.[49] From a systematic review and meta-analysis, Telang et al.[50] found no increased risk of PCa progression in males with a family history of PCa.[50] Although an increased incidence of PCa is found in males with first-degree familial history

of PCa, this does appear to cause an increase in the incidence of cancer progression in these individuals. However, Bratt et al.,[51] in a nationwide population-based study in the USA, found age-specific PCa incidences were much higher among males with family history of PCa and of high-risk cancer. It appears that the risk of PCa is 2–3 times higher among those with a family history of PCa and those cancers that can lead to mortality from PCa.

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

The authors hope this review will bring some clarity to the issues associated with PCa screening in Western nations. The issues associated with PCa screening in less developed countries are beyond the scope of this review. The following can be summarised:

1. In trying to balance between early diagnosis and avoiding unnecessary procedures or cost, the pendulum, in the past decade, has swung to the side of excessive avoidance of PCa screening. This has led to worsening of outcomes in PCa. This fact has been realised in recent years and efforts to correct this trend is underway. This review is part of that process.
2. PSA-based PCa screening is inadequate because of its lack of specificity; however, this may be the only option that is acceptable and available. The authors have come up with processes that may help overcome some of the shortcomings of PSA-based PCa screening (Figure 1 and Figure 2).
3. New biomarkers and molecular markers have shown promise in overcoming the defects of PSA-based screening in PCa (Table 2). However, the usefulness of each need to be robustly compared so that uniform guidelines for post-PSA-era PCa screening can evolve. Pharmaceutical and other private entities have no incentives to conduct clinical trials that would compare different commercial panels against each other. The National Cancer Institute (NCI)/National Institutes of Health (NIH), the National Health Service (NHS), and similar national health research or health agencies have to design new clinical trials to address this aspect of the PCa screening.
4. Special populations, such as African Americans, the elderly, or those with familial history of PCa, require special care and more specific, and perhaps separate, guidelines. The authors attempted such recommendations in this paper. These attempts should be considered a work in progress, and mean to act as catalyst for new clinical trials and to fill a void until post-PSA-era guidelines evolve.
5. Policy makers are urged to develop new clinical trials quickly to address the deficits related to the current state of PCa screening highlighted in this review paper. This will help save lives now and in the future, especially for special populations. These clinical trials should aim to balance the goals of saving lives from PCa mortality, avoiding unnecessary interventions and treatment, unnecessary cost associated with unnecessary interventions, and the outcome endpoints should include quality-of-life improvements. The clinical trials being designed should have sufficient power to address the issues related to the special populations.

Table 2: Summary of screening biomarkers in prostate cancer management.

Biomarker	Specimen	Clinical endpoints	Target patient population	Management guidelines	Agency and cost in the USA
PSA	Blood	Risk of HG cancer (score <4 ng/mL and >10 ng/mL)	Males ≥45 years with enlarged prostate; obese males; family history of PCa.	Score <4ng/mL: normal. Score 4–10 ng/mL: borderline. Score >10 ng/mL: 50% confirmed PCa.	FDA approved; covered by most insurance policies at $40 USD or without insurance at $39–53 USD.
PHI (prebiopsy)	Blood	Risk of HG cancer on biopsy (score 1–100).	Males ≥50 years with PSA 4–10 ng/mL and negative DRE results who are considering initial biopsy.	Score 0–26.9: 9.8% risk of HGD. Score 27–35.9: 16.8% risk of HGD. Score 36–54.9: 33.3% risk of HGD. Score ≥55: 50.1% risk of HGD.	FDA approved; covered by Medicare and most insurance policies at $80–100 USD or without insurance at $750 USD.
4K (prebiopsy)	Blood	Percentage risk of HG cancer on biopsy.	Males with elevated PSA/abnormal DRE results who are considering initial or repeat biopsy.	Low risk (1–7.5%): safe to defer biopsy with follow-up PSA. High risk (≥ 20%): perform biopsy.	CLIA certified; not covered by insurance at $395 USD.
mpMRI	N/A	To distinguish between benign and malignant PCa.	Males with elevated PSA/abnormal DRE results who are on active surveillance.	Can prevent 30% of males from unnecessary biopsy. Negative MRI: no HGD; positive MRI: risk of HGD.	FDA approved; covered by insurance policies at $500 USD or without insurance at $700–1,500 USD.
MiPS (prebiopsy)	Urine	Percentage risk of Gleason score ≥6 disease on biopsy; percentage risk of HG cancer on biopsy.	Males with elevated PSA/abnormal DRE results who are considering initial biopsy.	Dose not provide low and high risk cut-offs.	CLIA certified; not covered by insurance at $400 USD.
SelectMDx (prebiopsy)	Urine	Percentage risk of Gleason score ≥6 disease on biopsy; percentage risk of HG cancer on biopsy.	Males with elevated PSA/abnormal DRE results who are considering initial or repeat biopsy after initial negative results.	Low risk: routine follow-up and screening.	CLIA certified; limited Medicare coverage and not covered by insurance at $4,200 USD.
ExoDx (prebiopsy)	Urine	Percentage risk of Gleason score ≥7 disease on biopsy.	Males ≥50 years with PSA 2–10 ng/mL who are considering initial biopsy.	Score >15.6: increased risk of HG disease.	CLIA certified; covered by Medicare and most insurance policies at $395 USD or without insurance at $1,200 USD.
PCA3 (after negative biopsy)	Urine	Percentage risk of Gleason score ≥6 disease on biopsy (score 1–100).	Males >50 years who are considering repeat biopsy after initial negative biopsy.	Score 1–25: low risk of cancer (safe to defer biopsy). Score ≥26: high risk of cancer (repeat biopsy).	FDA approved; covered by most insurance policies at $385 USD or without insurance at $3,171 USD.

4K: Prostate Specific Kallikrein; CLIA: Clinical Laboratory Improvement Amendments; DRE: digital rectal examination; ExoDx: Intelliscore, nondigital digital rectal examination; FDA: U.S. Food and Drug Administration; HG: high grade (Gleason ≥7); HGD: high-grade disease; MiPS: Mi Prostate Score Urine Test; mpMRI: multiparametric MRI; PCA3: prostate cancer antigen 3 test; PHI: Prostate health index; SelectMDx: liquid biopsy.

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Cross-Sectional Study of Ethnicity and Chronic Heart Failure: Complex Interplay of Health and Wealth

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Disclosure: The authors have declared no conflicts of interest.

Received: 26.12.2018

Accepted: 03.01.2020

Keywords: Cardiomyopathy, epidemiology, heart failure.

Citation: EMJ. 2020;DOI/10.33590/emj/18-00121

Abstract

Objectives: Extrapolating data from international and regional registries on chronic heart failure provides a challenge in Malaysia in view of it being a multicultural country. This study aimed to illustrate the chronic heart failure landscape within northern Kuala Lumpur and identify differences amongst major ethnic groups.

Methods: A retrospective, single-centre study was conducted between the 1st January 2013 and 30th April 2016. Patients with left ventricular ejection fraction ≤45% were identified and information was collected on these individuals' demographics, risk factors, and aetiology. Comparisons were made between three major ethnic groups within Malaysia, and between the author's database and that of other international registries.

Results: 1,181 patients were identified, the majority being Malay (67.3%) and male (81.2%). The mean age was 58.6±11.4 years. The majority had ischaemic risk factors, including previous and current smoking habits (56.7%), coronary artery disease (61.5%), hypertension (66.6%), diabetes (57.7%), and dyslipidaemia (44.5%). There were significant differences noted when looking at rates of dyslipidaemia, diabetes, atrial fibrillation, and chronic kidney disease amongst different ethnicities. The mean left ventricular ejection fraction in Malays was lower (33.1±9.6%) compared to that of Chinese (34.7±8.7%) and Indians (34.9±8.3%). There was marked differences seen in this study's cohort, compared to three major Asian registries: The 2003 Chong et al. registry, Inter-CHF, and ASIAN-HF.

Conclusion: There exists great disparity in chronic heart failure burden amongst populations, and therefore local registries are needed to narrow the gap in knowledge regarding chronic heart failure within Malaysia.

INTRODUCTION

Heart failure affects up to 26 million adults worldwide.[1] The overall economic cost of heart failure in 2012 was estimated to be $108 billion per annum in the USA, of which 60% was related to inpatient care.[2,3] There has been increasing awareness over the last decade of the importance of developing heart failure registries to better understand the burden of the disease. Unfortunately, the majority of these registries

are focussed on Western populations.[4,5] With a population of over 600 million people within South East Asia, where the ethnicity and healthcare services are diverse, extrapolating data from pre-existing registries may not be appropriate, and there is a need for a dataset that reflects the impact of disease on the Malaysian population, specifically on various ethnic groups. Only a few collaborative international registries exist (e.g., ADHERE-AP, 2011; ASIAN-HF, 2016; Inter-CHF, 2016), which include 1,808 patients from Malaysia accumulatively: a gross under-representation of the Malaysian population as a whole.[6-8] This study aimed to illustrate the variation in chronic heart failure landscape within northern Kuala Lumpur and identify differences amongst the major ethnic groups in Malaysia. This study's dataset was compared with that of other Asian registries.

Heart Failure is a major burden in the majority of countries and registries allow us to better understand the affected populations. Differences in heart failure progression amongst various ethnic groups exist and yet are poorly documented. The multicultural landscape of Malaysia provides a foundation in which we can better understand the influence of socioeconomic factors in heart failure prognosis and outcomes. Understanding socioeconomic factors allows for better tailoring of disease management, as opposed to adopting a 'one size fits all' strategy in our population.

METHODS

A retrospective, single-centre study was conducted by reviewing all echocardiography reports available in the Cardiology Unit at the Universiti Teknologi MARA (UiTM) between the 1st January 2013 and 30th April 2016. The centre is a university-linked hospital with in-house cardiology services, offering both invasive and noninvasive treatments. Referrals are primarily from primary care and other hospitals, usually within the northern Kuala Lumpur region. Information on medication and prior investigations or interventions performed were not necessarily available unless provided by the referring centres. The cardiology unit consists of a team of cardiology consultants, registrars, and nursing professionals with training in cardiology care. Echocardiography is performed by both clinicians and cardiovascular scientists with recognised, in-house training. Services are audited annually to ensure quality assurance. External sources of echocardiography results were excluded from this study. The possibility of systematic difference would be kept minimal as training provided in-house reflects that of national standards.

Patients with a recorded left ventricular ejection fraction (LVEF) of ≤45%, with clinical symptoms and signs consistent with heart failure as determined by clinicians, were included in the study. Patients were contacted prior to data collection to allow for accurate data capture on mortality. Information on demographics, cardiovascular risk factors, and aetiology of heart failure were obtained from the corresponding electronic medical record. Data analysis was performed via IBM SPSS statistical software version 23.0© (Armonk, New York, USA), and comparison was made between the three major ethnic groups in Malaysia: Malay, Chinese, and Indian. Further analysis was performed by comparing results with that of other established registries. Descriptive statistics were used to summarise patient characteristics and other variables. Categorical data were presented by frequency and percentage. Continuous data were analysed using mean and standard deviation. Comparisons across different ethnic groups were analysed via chi square and ANOVA testing. Institutional ethics committee approval was obtained from the university, and no financial support was necessary for this study.

RESULTS

Comparison Between Ethnic Groups

An all-inclusive total of 1,181 patients with LVEF ≤45% were identified between the 1st January 2013 and 30th April 2016 (Table 1). The majority of patients were Malay (67.3%) and male (81.2%). When divided into different ethnic groups, male predominance was also seen (Table 1). The mean age was 58.6±11.4 years old. It was noted that Indians tended to experience heart failure at a younger age (57.8±0.4 years). Of note, more than half of patients in each ethnic group were aged 50–69 years old.

Slightly more than half (56.7%) of patients were smokers or ex-smokers, and the majority exhibited common ischaemic risk factors, including coronary artery disease (61.5%), hypertension (66.6%), diabetes (57.7%), and dyslipidaemia (44.5%).

Table 1: Baseline characteristics of chronic heart failure in north Kuala Lumpur.

	Total	Malay	Chinese	Indian	Other	p value
	(N=1,181)	(n=795)	(n=204)	(n=168)	(n=14)	
Male	81.2%	80.7%	84.3%	81.1%	69.2%	
Age						
Mean age	58.6±11.4	58.2±11.7	62.0±10.0	57.8±0.4	46.1±14.2	p<0.001
<40	65 (5.5%)	49 (6.2%)	4 (2.0%)	7 (4.2%)	5 (35.7%)	
40–49	176 (14.9%)	125 (15.7%)	22 (10.8%)	26 (15.5%)	3 (21.4%)	
50–59	359 (30.4%)	244 (30.7%)	52 (25.5%)	59 (35.1%)	4 (28.6%)	
60–69	386 (32.7%)	245 (30.8%)	81 (39.7%)	59 (35.1%)	1 (7.1%)	
70–79	168 (14.2%)	112 (14.1%)	39 (19.1%)	16 (9.5%)	1 (7.1%)	
>80	27 (2.3%)	20 (2.5%)	6 (2.9%)	1 (0.6%)	0 (0.0%)	
Heart rate (bpm)	74.0±14.0	74.8±15.0	72.6±13.7	76.6±15.0	80.9±15.6	p<0.05
Blood pressure (mmHg)						
Systolic	128±22	129±23	125±20	124±19	125±24	p>0.05
Diastolic	76±14	77±14	74±13	74±13	77±14	p>0.05
Smoking status						
Smoker	228 (19.3%)	145 (18.2%)	49 (24.0%)	33(19.6%)	1 (7.1%)	
Ex-smoker	349 (29.6%)	251 (31.6%)	46 (22.5%)	50 (29.8%)	2 (14.3%)	
Medical history						
CAD	726 (61.5%)	475 (59.7%)	124 (60.8%)	118 (70.2%)	9 (64.3%)	p>0.05
Hypertension	786 (66.6%)	527 (66.3%)	131 (64.2%)	122 (72.6%)	6 (42.9%)	p>0.05
Diabetes	681 (57.7%)	440 (55.3%)	109 (53.4%)	128 (76.2%)	4 (28.6%)	p<0.0001
Dyslipidaemia	526 (44.5%)	363 (45.7%)	77 (37.7%)	83 (49.4%)	3 (21.4%)	p<0.05
CKD	163 (13.8%)	126 (15.8%)	26 (12.7%)	11 (6.5%)	0 (0.0%)	p<0.05
Stroke	78 (6.6%)	55 (6.9%)	14 (6.9%)	9 (5.4%)	0 (0.0%)	p>0.05
Hyperthyroidism	20 (1.7%)	14 (1.8%)	1 (0.5%)	4 (2.4%)	1 (7.1%)	p>0.05
Atrial fibrillation	86 (7.3%)	70 (8.8%)	11 (5.4%)	5 (3.0%)	0 (0.0%)	p<0.05
COPD	20 (1.7%)	12 (1.5%)	3 (1.5%)	4 (2.4%)	1 (7.1%)	p>0.05
LVEF (%)						
Mean		33.1±9.6	34.7±8.7	34.9±8.3	36.6±8.9	p<0.05
40–45	419 (35.5%)	270 (34.0%)	81 (39.7%)	61 (36.3%)	8 (57.1%)	p>0.05
25–39	553 (46.8%)	369 (46.4%)	96 (47.1%)	83 (49.4%)	5 (35.7%)	p>0.05
<25	104 (8.8%)	154 (19.4%)	25 (12.3%)	24 (14.3%)	1 (7.1%)	p>0.05
Aetiology						
Ischaemic heart disease	726 (61.5%)	475 (59.7%)	124 (60.8%)	118 (70.2%)	9 (64.3%)	p>0.05
Valvular heart disease	412 (34.9%)	290 (36.5%)	66 (32.4%)	50 (29.8%)	6 (42.9%)	p>0.05
Cardiomyopathy	39 (3.3%)	28 (3.5%)	7 (3.4%)	4 (2.4%)	0 (0.0%)	p>0.05
Thyrotoxicosis	20 (1.7%)	14 (1.8%)	1 (0.5%)	4 (2.4%)	1 (7.1%)	p>0.05
Arrhythmias	39 (3.3%)	34 (4.3%)	5 (2.5%)	0 (0.0%)	0 (0.0%)	p>0.05

bpm: beats per minute; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; LVEF: left ventricular ejection fraction.

Table 2: Demographic comparison with other Asian based registries.

	North Kuala Lumpur	Chong et al.[9]	ASIAN-HF	Inter-CHF
	Heart Failure Registry	2003	2016	(Asia)
	2017	(N=97)		2016
	(N=1,181)		(N=5,276)	(N=1,253)
Male	81.2%	80.7%	78.2%	69.0%
Mean age	58.6	53.6	59.6	58.5
Blood pressure (mmHg)				
Systolic	128±22	N/A	118.4±20.2	N/A
Diastolic	76±14	N/A	72.4±12.6	N/A
Smoking status				
Smoker/ex-smoker	56.7%	22.7%	45.0%	35.9%
Medical history				
CAD	61.5%	49.5%	50.2%	26.3%
Hypertension	66.6%	49.5%	51.9%	55.3%
Diabetes	57.7%	28.9%	40.4%	32.7%
Dyslipidaemia	44.5%	N/A	N/A	25.1%
CKD	13.8%	N/A	N/A	8.23%
Stroke	6.6%	N/A	6.4%	4.37%
Hyperthyroidism	1.7%	1.0%	N/A	N/A
Atrial fibrillation	7.3%	4.1%	17.9%	N/A
COPD	1.7%	12.4%	8.3%	N/A
Mean LVEF (%)	33.7±9.3	N/A	28.0%	N/A
Causes of heart failure				
Ischaemic heart disease	61.5%	49.5%	47.0%	48.0%
Valvular heart disease	34.9%	4.1%	N/A	12.0%
Cardiomyopathy	3.3%	4.1%	N/A	11.0%
Thyrotoxicosis	1.7%	1.0%	N/A	5.0%
Arrhythmias	3.3%	N/A	N/A	N/A
Hypertension	N/A	18.6%	N/A	14.0%
Number of patients from Malaysia (n)	1,181	97	541	362

ASIAN-HF: Asian Sudden Cardiac Death in Heart Failure; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; Inter-CHF: International Congestive Heart Failure; LVEF: left ventricular ejection fraction; N/A: not available.

When comparisons were made between ethnic groups, there were significant differences in the rate of diabetes, for which Indians ranked highest (76.2%), followed by Malays (55.3%) and Chinese (53.4%). The prevalence of atrial fibrillation (AF) in this cohort was low, at 7.3%. When analysing individual ethnic groups, Malays presented more commonly with AF (8.8%) as opposed to Chinese (5.4%) and Indians (3.0%). The prevalence of chronic kidney disease was significantly higher in Malays (15.8%) versus Chinese (12.7%) and Indians (6.5%). The majority of patients, as a whole (61.5%) and within individual ethnic groups (59.7%, 60.8%, and 70.2% of Malays, Chinese, and Indians, respectively), presented with ischaemia-related heart failure.

Table 3: Comparison of major ethnicity in Malaysia versus ASIAN-HF cohort.

	Malay (n=795)	Malay* (n=810)	Chinese (n=204)	Chinese* (n=1,561)	Indian (n=168)	Indian* (n=1,649)
Male	80.68%	81.4%	84.3%	80.8%	81.1%	76.1%
Mean age	58.2±11.7	56.4±11.4	62.0±10.0	60.8±13.5	57.8±0.4	58.1±12.2
Heart rate (bpm)	74.8±15.0	N/A	72.6±13.7	N/A	76.6±15.0	N/A
Blood pressure (mmHg)						
Systolic	129±23	121±21	125±20	120±20	124±19	116±19
Diastolic	77±14	81±17	74±13	72±12	74±13	73±11
Smoking status						
Smoker/ex-smoker	57.4%	61.1%	55.9%	54.1%	56.8%	66.1%
Medical history						
CAD	59.7%	49.8%	60.8%	54.2%	70.2%	59.8%
Hypertension	66.3%	62.1%	64.2%	57.5%	72.6%	42.5%
Diabetes	55.3%	50.2%	53.4%	40.9%	76.2%	42.1%
Stroke	6.9%	7.4%	6.9%	7.4%	5.4%	3.0%
Atrial fibrillation	8.8%	14.1%	5.4%	23.1%	3.0%	4.7%
COPD	1.5%	9.6%	1.5%	9.9%	2.4%	5.2%
Mean LVEF (%)	33.1±9.6	25.0±5.0	34.7±8.7	28.0±6.0	34.9±8.3	28.0±4.0
Aetiology						
Ischaemic heart Disease	59.7%	66.8%	60.8%	46.9%	70.2%	42.9%
Valvular heart disease	36.5%	N/A	32.4%	N/A	29.8%	N/A
Cardiomyopathy	3.5%	N/A	3.4%	N/A	2.4%	N/A
Thyrotoxicosis	1.8%	N/A	0.5%	N/A	2.4%	N/A
Arrhythmias	4.3%	N/A	2.5%	N/A	0 .0%	N/A

*These data were taken from the Asian-HF registry.

ASIAN-HF: Asian Sudden Cardiac Death in Heart Failure; bpm: beats per minute; CAD: coronary artery disease; CKD: chronic kidney disease; COPD: chronic obstructive pulmonary disease; LVEF: left ventricular ejection fraction; N/A: not available.

The mean echocardiographic LVEF in Malays (33.1±9.6%) was lower compared to that of Chinese (34.7±8.7%) and Indians (34.9±8.3%). Systolic and diastolic blood pressure (129±23, 125±20, and 124±19 mmHg in Malays, Chinese, and Indians, respectively; p>0.05) and average heart rate (74.8±15, 72.6±13.7, and 76.6±15 beats per minute in Malays, Chinese, and Indian, respectively; p>0.05) varied little across ethnicities.

Comparison Between Registries

Comparison was made between this study's registry, and three others: Chong et al,[9] Inter-CHF,[8] and ASIAN-HF,[7] with varying proportions of Malaysian patients being included in each (Table 2 and 3). There was a higher proportion of males in this study's cohort. The mean age of heart failure amongst the Malaysian population is comparable to other Asian-based registries, which is between 58 and 60 years of age.[7,8] Interestingly, Chinese patients tend to be older in both this study's cohort and the Asian-HF registry.

Age of heart failure onset was at least a decade younger in this study's cohort versus European patients.[4] This disparity may be due to the higher prevalence of physical inactivity, smoking, and obesity in this population which leads to the development of heart failure sooner as seen more commonly in youths. This is on top of pre-existing evidence of a higher prevalence of cardiovascular risk burden in Malaysia amongst other South-East Asian countries.[10-12] This is illustrated by this study's cohort, showing a higher prevalence of smoking (56.7%), coronary artery disease (61.5%), hypertension (66.6%), diabetes (57.7%), dyslipidaemia (44.5%), chronic kidney disease (13.8%), and stroke (6.6%). Smoking was also seen to be less prevalent amongst Chinese patients compared to both Malays and Indians in this study, which may suggest not only an ethnicity component, but also environmental, cultural, and social elements linked to disease distribution.

The Malaysian prevalence of AF (7.3%) was lower in comparison to the ASIAN-HF cohort. This study's findings are consistent with other Malaysian-based datasets, such as that of Lim et al.,[13] in which overall prevalence of AF was 0.54%, lower than the global average of 1.00%.[13] Also noted was that this study's average LVEF was much lower (33.7±9.3%) than compared with Inter-CHF (28.0%), likely due to the heavy ischaemic burden noted in the Malaysian population which is greatly linked to heart failure with a reduced ejection fraction phenotype.[14] When compared to the Inter-CHF group, despite lower left ventricular systolic function, mean systolic and diastolic blood pressure were roughly 10 and 5 mmHg higher, respectively, in this study's cohort. This is likely explained by a high prevalence of coexisting hypertension.

DISCUSSION

This is the largest reported heart failure population in Malaysia. It gives a unique perspective of the population in the north of Kuala Lumpur, with its multi-ethnic background. The distribution of patients within the sample population was comparable to that of Malaysia's demography, with a Malay population rate of 69.1%, followed by 17.3% Chinese and 14.2% Indian.[15]

Income variation plays an important role is disease variance, as illustrated by the World Health Organization (WHO) Global Status Report 2014 revealing lower rates of physical inactivity, smoking, overweightness, impaired blood glucose, and raised blood pressure in high-income generating countries (such as the UK and USA, as well as Brunei and Singapore within the South-East Asian region). The converse is true for countries like Indonesia, Thailand, and Malaysia.[11] Interestingly, income variation and disparity exist even between ethnic groups within a country, including that of Malaysia. Data support the fact that those of Chinese ethnicity generated the largest per capita income compared with those of Malay and Indian descent (1.49 versus 1.11 versus 1.24, respectively).[16] This may also partly explain the lower rates of smoking amongst Chinese in this study's cohorts, where an inverse relationship between income generation and tobacco use exist.[17] Of note, due to a lack of data, the authors were unable to comment on differences in delivery of care, including medication prescription or monitoring of risk factors and heart failure progression, amongst different ethnic groups.

Research has shown the important role of ethnicity in disease outcome. Although genetic factors have been shown to be an important risk factor, the interdependency between socioeconomic characteristics, behavioural characteristics, and access to quality healthcare does exist and cannot be completely excluded. For example, the MESA, V-HeFT 1, and SOLVD trials found differences in cardiovascular burden and heart failure progression amongst different ethnic groups, based on differences in heart failure aetiology, neuro–hormonal pathway stimulation, and the pharmacological treatments administered.[18-20]

It is important to discuss the burden of ischaemia, especially in the context of heart failure, as the majority (as seen in this study's cohort) of

chronic heart failure cases are ischaemia driven (Table 1). Ethnicity-based disparities in cardiovascular risk factors and coronary artery disease have previously been reported by studies carried out in Singapore.[21,22] The young, male, and both Indian and Malay-predominant demographics in this study's population do not only mirror the existing demography of a Malaysian population as a whole, but also reflect the distribution of ischaemic heart disease burden within the population. The high proportion of patients with ischaemic risk factors, evidenced further by Malaysia's national ischaemic heart disease database, supports this.[23] The male preponderance was expected, with established evidence highlighting ischaemia as being more prevalent in males and post-menopausal women.[24] With regards to ethnic distribution, there is also evidence supporting a high proportion of ischaemia amongst Malays and Indians, as seen in both local (Zuhdi et al.[25] and Hoo et al.[26]) and regional studies (Wong et al.[27]). Furthermore, studies have previously reported a higher prevalence of obesity and poor rate of engagement in physical activity amongst those of Malay and Indian descent.[28]

Limitations

Unfortunately, without information of the actual population within Malaysian locally and nationally, it is often difficult to interpret differences between ethnic groups and socioeconomic status. Furthermore, any differences in age, sex, and ethnicity might also be reflective of survivor bias, which is difficult to exclude in this study. However, this study's aim was to highlight the lack of such data and hopefully spur interest in collaborative efforts to develop local and national registries on chronic heart failure.

Implications from the study

This study highlights the uniqueness seen in a multi-ethnic population, which prior to this has not been demonstrated before. Although studies have been done in Malaysia's neighbouring country Singapore, results from those studies vary greatly and therefore are not extrapolatable to that of the Malaysian population, again illustrating the need for more local data on chronic heart failure.[7] The data obtained will pave the way for establishing more regional and national registries to better understand the disease.

CONCLUSION

Registries are important to better understand the landscape of heart failure amongst populations. However, appreciation is needed for the great heterogeneity within populations that may or may not be best represented from an accumulative, large database such as that of Asian-HF or Inter-CHF. Even amongst neighbouring countries, for example Singapore and Malaysia, with a closely linked population demography because of historical and political factors, there exists a great disparity in disease burden and risk factors associated with disease development and progression. As the prevalence of heart failure increases, with an increase in survivorship amongst those affected by ischaemic cardiomyopathy, the development of local registries that take into account differences in cultural and economic factors are essential for future service development purposes.

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