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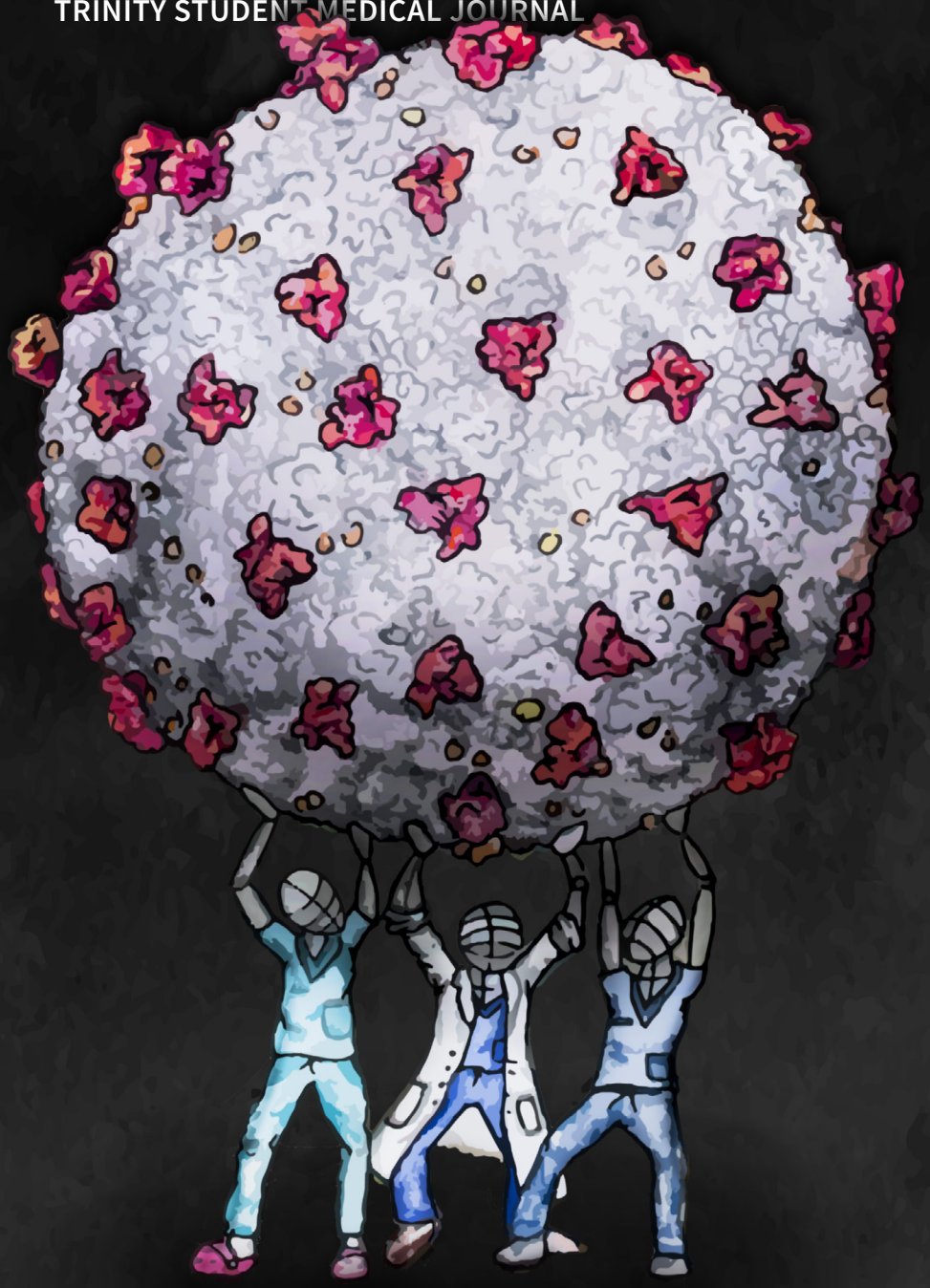

Resilience in Student Research

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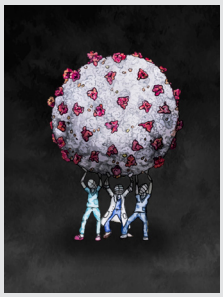

◄ **"A PROBLEM SHARED" BY BLÁITHÍN THOMAS**

Weltschmerz: a feeling of melancholy or world-weariness.

Although this may be the first time many of you readers are hearing this word, this feeling is a good descriptor for the emotions felt by people today.

The COVID-19 pandemic—and its contribution to an already insidious mental health crisis amongst healthcare workers—has left many of us feeling like we carry the weight of the world on our shoulders. However, we should never forget that we don't carry this weight alone, and that by coming together as colleagues and friends, we can overcome any obstacle.

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Volume 21 (2021) Editorial Team & Contributors

EDITOR'S FOREWORD

Resilience in Student Research

Noor Adeebah Mohamed Razif

School of Medicine, Trinity College Dublin, University of Dublin, Ireland (MohameNo@tcd.ie)

Since early 2020, the world witnessed SARS-CoV-2—the COVID-19 virus—taking away the lives of their loved ones and stopping many large in-person gatherings such as going to the cinemas, conferences, and in-person lectures. Research done by students was also one of the activities that was inevitably affected by the pandemic. Many aspiring student researchers faced a halt to their exciting scientific investigations for the remaining academic year and their upcoming summer break unless they were essential or related to COVID-19. Various organised studentships and research programmes had to be cancelled too. This is largely because this research requires physical presence in a laboratory or physical interaction with patients. However, this did not bring student researchers down—their resilience shined through. In the past year, students around the world managed to make the most out of lockdowns and restrictions by carrying out research in the most accessible way they could, as demonstrated by the contents of this 21st volume of the TSMJ.

More importantly, it highlighted the various forms of research that could be done remotely. Literature reviews provide an overview of a topic using previously published works. The methodology of a literature review can be done at home since most publications are now readily available online. In this volume, Anurag Nasa wrote an insightful article about bacteriophage therapy and its potential to be a more sustainable alternative to antibiotic treatment for infections. The use of bacteriophage therapy may be useful to counter multi-drug resistant bacteria that are on the rise globally. Matthew Thomas, on the other hand, reflected on the potential of using bacteria as a vector for drug delivery, particularly with the use of synchronised lysis circuits, a niche form of communication that bacteria use to coordinate activities in a population. He also noted the incorporation of nanobodies into these bacteria, particularly those that exhibit immune checkpoint inhibitor properties, which makes drug delivery more efficient and reduces the toxicity of the medication. Aidan O'Riain provided a comprehensive review of adenovirus vectors, which have already been used to develop one of the COVID-19 vaccines, and its future potential in clinical practice. Literature reviews are an excellent starting point for researchers and health practitioners to understand a concept better.

Systematic reviews are one of the highest levels of scientific evidence based on the Oxford Centre for Evidence-Based Medicine[1]. They synthesise information from multiple existing sources in an organised approach, within a specific set of parameters, to produce findings that are reliable with minimal bias. Not to mention, it is also a type of research that is convenient to do in this pandemic, as most of the work is done remotely by online database search and by video call meetings among co-authors. Sarah Waicus and Lauren Vrbanic conducted a systematic review of the treatment of hydrocephalus in congenital toxoplasmosis using the known drugs, pyrimethamine and sulfonamide. Roisin Guihen and co-authors managed to determine the common risk factors and associated comorbidities of hidradenitis suppurativa. Stefan Elekes and colleagues provided an up-to-date analysis of studies about the latest screening and treatment options for latent tuberculosis infection. Additionally, the rapid studies published for COVID-19 research also provided an excellent source of data for systematic reviews to be conducted from, even by students. Laith Al Azawi and colleagues studied the efficacy of the drug dexamethasone in patients who were admitted to intensive care units due to COVID-19, based on recent evidence. Systematic reviews are vital in informing clinical decision making in today's practice.

Various medical specialty bodies frequently hold essay competitions not only to promote their field, but also to encourage students to enhance their knowledge in the specialty. Niall O'Rourke, runner up of the College of Psychiatrists of Ireland Medical Student and Intern Essay Prize 2021, provided a succinct feature on virtual therapies that currently exist in psychiatry. He pointed out exciting new virtual therapies

that may have potential, although numerous limitations still need to be addressed before these therapies enter common clinical practice. Meanwhile, Lowri Edwards summarised the importance of patient-centred care by anaesthesiologists and other healthcare workers to manage a patient's expectations throughout the process of a procedure or operation. This was submitted to the College of Anaesthesiologists of Ireland 7th Annual Medical Student Essay Competition 2020. Garrett Huwyler provided a comprehensive discussion and comparison about the regulation of non-surgical cosmetic interventions in the European Union and the United Kingdom that earned him the prize winner of the TCD Dermatology Society Essay Prize 2021. Essays may not seem to be a valued form of scientific literature, but their key messages are usually relevant and suitable for the public to comprehend due to their straightforward evidence-based content and language.

To conclude, students should use the opportunity of this pandemic to explore other forms of research, particularly essays and reviews. Essay competitions are an ideal avenue to sharpen one's research skills. Reviews should not be underestimated and although they are not experimental or patient-based research, an enormous amount of effort and collaboration is still needed. This pandemic also highlights the need to incorporate research like this into the curricula of medical schools as it can have tangible benefits for students, such as increasing their depth of knowledge, improving scientific writing skills, and providing the chance to publish their works. Embedding research skills in students during their undergraduate studies better prepares them to carry out their own research in the future. More importantly, these skills will help them to translate and apply the knowledge gleaned from published works into their future occupations. ◄

Declarations

The author declares no conflicts of interest.

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I would like to thank the School of Medicine of Trinity College Dublin for not only helping us in publishing our 21st volume but also for encouraging students to carry out research by embedding research projects into the curriculum. I am also humbly grateful for sponsorship from the Medical Protection Society, AMBOSS, and the Trinity Association and Trust.

Lastly, I would like to express my deepest gratitude and appreciation towards the whole TSMJ committee for their unwavering support and for their hard work in making this volume possible. We could not have done this without all of you.

PERSPECTIVE

College of Psychiatrists of Ireland Medical Student and Intern Essay Prize 2021 Runner Up

Optimising Mental Health in a Virtual World – Connecting from a Distance

Niall O'Rourke

School of Medicine, Trinity College Dublin, University of Dublin, Ireland (ORourkN4@tcd.ie)

Key Points

- The use of modern technology and virtual approaches to mental health therapy is growing in importance across a diverse range of disorders.
- While existing mental health therapies have been successfully adapted to virtual modes of delivery, modern technology is also driving exciting novel therapies.
- Studies suggest virtual approaches to mental health care have both advantages and disadvantages. Further study and a cautious, evidence-based approach is key.
- The complex and co-morbid nature of psychiatry means that patients will continue to require assessment and management by specialists. Virtual therapies are useful components of a stepped-care model, however patients with severe mental health problems will continue to require in-person care.

Keywords: Mental health, Technology, Internet-based cognitive behavioural therapy (iCBT), Virtual therapy, Evidence-based psychiatry

Introduction

Our modern world and its consequences for mental health have been extensively discussed of late[1]. While some studies have highlighted the negative aspects of modern technologies such as social media[2], others have focused on the positives[3]. The question of how we can optimise our mental health in a fast-changing world is an important one. In this essay, I will explore where we stand with technology and mental health, outline some exciting new developments, and ultimately seek to address how we can optimise mental health in a virtual world.

Virtual Cognitive Behavioural Therapy (CBT)

Since its development in the 1960s, Cognitive Behavioural Therapy (CBT) has become a mainstay of mental health treatment[4]. There is now a wealth of evidence for its effectiveness in the treatment of depression[5], anxiety disorders, and other mental health disorders[4]. Despite this, many patients with mental health disorders remain untreated[6]. The potential for modern technology to broaden patient access to CBT has been recognised for almost 20 years, with the development of internet-based CBT (iCBT) programmes[4]. There is now good-quality evidence to support the effectiveness of iCBT in both adults[7] and children[8,9]. The ability of virtual therapies to improve access to mental health treatment is therefore well-established, but has become even more evident during the COVID-19 pandemic. In November 2020, Ireland's Health Service Executive (HSE) published its online *Mind Your Wellbeing* programme[10]. This is a virtual, video-based wellbeing programme consisting of 5 sessions. In the UK, the National Health Service (NHS) promotes the delivery of virtual CBT-based therapy via smartphone apps[11] such as *Calm Harm*[12] and *ThinkNinja*[13]. Besides offering improved public access to CBT, virtual therapies have the added advantages of greater cost-effectiveness than face-to-face therapy and greater potential for patient retention[14]. While virtual CBT and CBT-based therapies have become accepted approaches in the management of depression and anxiety disorders, virtual approaches in other mental health disorders are less well-established[14]. However, mental health means much more than just depression and anxiety. We must also look at how technology can be applied to other disorders, if we are to fulfil its potential in advancing mental health in our modern world.

Other Virtual Therapies

It is becoming clearer that there is indeed potential for virtual therapies beyond depression and anxiety. Recently, there have been small studies supporting virtual Acceptance and Commitment Therapy (ACT) in the management of somatic symptom disorders such as fibromyalgia[15,16]. Another area of research of late has been in the development of Virtual Reality Exposure Therapy (VRET) for the treatment of phobias[17]. While these are examples of how existing treatments (i.e. ACT and exposure therapy) can be adapted to new technologies, there are also exciting novel approaches to virtual mental health therapies. The front-cover of Nature issue 7465 was emblazoned with the words "GAME CHANGER", heralding the first video game

developed as a medical intervention[18]. Playing this video game, *NeuroRacer*, resulted in cognitive improvements in a cohort of older adults[19]. In June 2020, the US Food and Drug Administration (FDA) authorised *EndeavorRx*, the first video game-based therapeutic[20], which is designed to treat inattention in children with attention-deficit/hyperactivity disorder (ADHD). In a randomised control trial, this game demonstrated improvements in symptoms of ADHD in a cohort of children aged 8–12 years[21]. A recently published randomised control trial of a different, mindfulness-based video game also demonstrated improvements in cognition and behaviour in a cohort of adolescents who had suffered adverse childhood experiences[22]. However, these developments are not without controversy: a 2015 meta-analysis of similar cognitive training approaches found minimal benefits in children and adolescents with ADHD[23]. As the exciting new field of virtual mental health therapies develops, it is important to apply the rigorous scientific method and not overstate the benefits of novel therapies[24]. While virtual therapies show promise for many mental health disorders, there is still a strong case for an approach utilising cautious optimism and evidence-based medicine.

Current Issues with Virtual Approaches

A recurrent theme in the literature surrounding virtual approaches to mental health is the need for further research[14,16,24]. While there are clear advantages to virtual therapies such as improved access and cost-effectiveness[14], there are also disadvantages. To truly optimise virtual approaches to mental health care, we must recognise these disadvantages and address them. To date, most of the research on virtual mental health treatments has focused on CBT[25]. Two recent meta-analyses have found lower rates of deterioration among patients who received iCBT than controls[26,27], and it appears that these rates of deterioration are similar to patients who receive face-to-face treatments[28]. However, another meta-analysis found that there was a significantly higher dropout rate among patients receiving iCBT compared to patients receiving face-to-face therapy[29]. There remains a lack of research on other negative outcomes from virtual therapies, and there is little research on emerging virtual therapies for disorders other than depression and anxiety[25]. There is also evidence that some patients are less willing to engage with internet-based therapy: a 2010 study in the US found that 91.9% of patients would consider face-to-face therapy, while only 48% would consider internet-based therapy[30]. Unsurprisingly, older patients were less willing to engage with internet therapy than younger patients.

To date, most virtual therapies are targeted at specific disorders[14]. However, comorbidities in patients with mental health disorders is common. For example, mood and anxiety disorders commonly present together[31,32]. Virtual therapies may be more suited to some patients, and less suited to others; patients with severe mental health problems will continue to require face-to-face services. Recent studies have sought to address how internet-based mental health therapies can be integrated into existing care systems; for example, as a step in a stepped-care model[33,34]. At a September 2018 conference titled "Changing Direction: Augmenting Mental Health Solutions", Dr. John Hillery, President of the College of Psychiatrists of Ireland, emphasised the continued need for trained specialists to assess and manage patients, regardless of the technology being used[35]. The many complexities and comorbidities seen in mental health underpin the need for highly trained and well-funded specialists to be at the forefront of implementing exciting new technologies. A careful, evidence-based approach led by experts is key.

Conclusion

In summary, virtual approaches to mental health therapy are becoming more and more accepted. Exciting new technological developments have made many novel interventions in a variety of mental health disorders possible. These are exciting times for mental health, and the importance of new technologies will likely grow in years to come. Moving forward, we need to have research and ongoing discussions about the advantages and disadvantages of different strategies and an evidence-based, expert-led approach. These considerations will allow us to make steady progress towards the goal of optimising mental health in a virtual world. ◄

Declarations

This essay was originally submitted as part of the College of Psychiatrists of Ireland Medical Student and Intern Essay Competition. The author declares no conflicts of interest.

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PERSPECTIVE

Anaesthetic Safety: What Do Patients Understand and Expect?

Lowri M. Edwards

School of Medicine, Trinity College Dublin, University of Dublin, Ireland (LEdwards@tcd.ie)

Key Points

- Patients' satisfaction depends on their prior expectations. These, in turn, are influenced by their understanding of a procedure.
- Provision of adequate pre-operative information can improve patients' perioperative outcomes.
- Patients' understanding of information provided may be optimised by employment of techniques including consolidation by multiple team members throughout the pre-operative period and the presence of a companion during consultations.
- A relative lack of awareness surrounding the anaesthesia and the role of the anaesthesiologists are contributing factors towards patient anxiety.
- A careful balance must be obtained between providing enough information to facilitate informed decision-making without causing unnecessary stress.
- Causes of patients' perioperative anxiety are variable and are often underestimated. Efforts to acknowledge and alleviate this anxiety are known to have therapeutic benefit.
- Patients' expectations of anaesthesia are influenced by a multitude of individual, social and cultural factors. This highlights the importance of meaningful discussion with the patient and a tailored approach to the pre-operative consenting procedure.
- In light of the COVID-19 pandemic, the good health of healthcare workers is increasingly being recognised as a priority for ensuring patient safety.

Keywords: Anaesthetics, Anaesthesiology, Informed consent, Patient expectations, Patient satisfaction, Patient-centred care

The term *expectation* is defined as "a belief that something will happen because it is likely" [1]. In order for a patient to have reasonable expectations, they must first have a clear understanding of the procedure, what can and cannot realistically be achieved by it, and what can go wrong with it. *Satisfaction* refers to the "degree of congruence between expectation and accomplishment"[2]. It therefore follows that a patient who lacks the necessary information to formulate a realistic expectation is unlikely to be satisfied by the outcome, regardless of the competence of the physician or the quality of care given.

Two types of expectation are relevant here. The first is conscious and acknowledged, whilst the other is often unrealised until it has not been met. When managing another's expectations, one can either explicitly set out to meet them, or alternatively, focus on regulating excessive anticipation. The latter can be facilitated by establishing a doctor–patient partnership in which there are shared responsibilities for ensuring the best possible outcome[3,4].

Properly informing all patients undergoing anaesthesia can be best achieved through a collaborative periprocedural process involving both the patient and healthcare workers[4]. The benefits of this approach are impressive. This process is necessary from a legal perspective to properly obtain genuine informed consent, but it is also recognised that patients' perception of their care and involvement in this are factors that also influence their perioperative state, and their sense of safety[5]. Studies demonstrate that the provision of adequate information regarding anaesthesia results in improved satisfaction, reduced pain levels, shorter hospital stays, and decreased anxiety, resulting in a reduced need for sedation[6].

It is also helpful to understand that expectations change based on the information given. Consent must, therefore, never be rushed, except in rapidly emergent and life-threatening circumstances. Patients' interpretation can often differ from the intended meaning. Ensuring that the message is consolidated by multiple team members is an effective way to reduce the extent to which this happens. Within an appropriate

confidentiality framework, having a companion present during the consenting procedure improves patients' active participation in the conversation and minimises misinterpretation by an unaccompanied anxious patient[7]. Even in the COVID-19 era, this is worth bearing in mind, and may be safely supported by means of telemedicine consultation.

When examining risk, it is understood that surgical patients either do not properly consider anaesthesia, or their anxiety is disproportionate. Both reactions stem from a lack of awareness regarding anaesthesia itself, and the extent of the anaesthesiologist's role. Studies have shown that as few as 52% of patients were aware that anaesthesiologists are qualified doctors[8], only 30% recognised that anaesthesiologists are involved in postoperative patient management[9], and 76% of patients did not realise that their survival could depend entirely on the anaesthesiologist[10]. These figures suggest that there is a responsibility to ensure that patients fully comprehend the roles of anaesthesia and the anaesthesiologist prior to consenting.

Irwen et al. reported that 90% of patients preferred to be informed of all potential complications, regardless of their severity[10]. While many may agree, it is also important to acknowledge individual factors that may contribute to anxiety levels. A balance must be achieved: on one hand, providing enough information to facilitate informed decision-making, while on the other, avoiding causing undue stress that could potentially cause patients to opt out of important procedures[11]. Burkle et al. reported that a majority of patients believed that common, less severe, and rare but highly consequential complications should all be disclosed, and that discussion should not be limited based on patients' suspected or apparent inability to comprehend the complexities of their care[12,13].

Anxiety is both an important and complex consideration in anaesthetics. Perioperative anxiety is known to correlate with greater requirement for anaesthetic, higher levels of postoperative pain, increased incidence of nausea and vomiting, and longer hospital stays[14]. Despite this, Badner et al. reported that anaesthesiologists tend to consistently underestimate patients' anxiety[15]. One study found that the commonest causes of perioperative anxiety included postoperative pain, regaining of consciousness or sensation during the surgery, dislike of needles or invasive procedures, and fear of death[14]. While these fears are understandable, it is the doctor's role to reassure and provide factual information to ensure that the patient's concern is proportionate to the actual risk.

Generally, lower anxiety levels correlate with increased age[16,17] and male sex[16], but curiously there is no relationship with education level[14,17], or prior experience[14,16]. Whilst managing patient expectations, anaesthesiologists could usefully attempt to understand and address factors and individual circumstances which contribute to patients' anxiety. The impact of alleviating this stress is so potent that it has been likened to "a dose of morphine"[16].

Much like anxiety, patients' wider expectations of anaesthesia are influenced by multiple factors. These vary depending on the patient's age, attitudes, type of surgery or anaesthesia, prior experience, risk profile, and level of suffering, among other influences. Cultural norms also play a substantial role in determining patient satisfaction. For example, one study highlighted significant differences in the anticipation and acceptance of pain levels reported between American and Vietnamese patients following highly similar procedures and methods[18]. Consideration of the family's expectations is also important, especially in the care of paediatric and elderly patients. Given these complexities therefore, it would be difficult for physicians to reliably predict patients' specific expectations without direct and meaningful discussion. Ticking boxes on a pre-op consent form falls short of what the current evidence indicates is good and necessary practice.

The recent COVID-19 pandemic has exposed a previously unrecognised expectation from healthcare workers—the expectation of their own good health. Anaesthesiologists and their colleagues on the front line have faced significant exposure not only to the virus itself but also the indisputable mental health care burden that has ensued[19]. Recognition of the impact of fatigue and poor health on performance has motivated the appreciation of healthcare worker safety as a priority for ensuring patient safety[19,20].

Care that is truly patient-centred is focused on incorporating the individual needs and expectations of patients into the provision of healthcare[6]. Patients can reasonably expect their anaesthesiologist to be healthy, competent, respectful, truthful, professional, to have regard for autonomy, and to act in their best interest. These expectations are universal to all healthcare professionals. Beyond this, patients should also expect an honest and bipartisan conversation resulting in an agreement on mutual and realistic goals, rather than one restricted to specific outcomes. "*In somno securitas*", the motto of the Association of Anaesthetists of Great Britain, refers not only to the physical safety of the anaesthetised patient, but also the peace of mind that results from true patient-centred perioperative care[21]. ◄

Declarations

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PERSPECTIVE

TCD Dermatological Society Essay Competition 2021 – Winner

Unnecessary or Negligent? A Look into the Regulation of Non-Surgical Cosmetic Intervention in Europe

Garrett Huwyler

School of Medicine, Trinity College Dublin, University of Dublin, Ireland (GarrettHuwyler@gmail.com)

Abstract

The regulation of medical devices and associated procedures is common across the globe, with country and regional variations directly impacting patient safety and ease of access. When considering non-surgical cosmetic interventions within Europe, the variations seen between member states of the European Union and that of the United Kingdom are quite dramatic. These regulations encompass procedures such as dermal fillers, botulinum toxin injections, and the application of lasers for skin rejuvenation treatments. Currently, the regulations in the European Union place an emphasis on quality control and safety for the products used by classifying them as medical devices and enforcing medical licensing requirements for their application. In contrast, the United Kingdom lacks regulation around both quality control and licensing requirements, placing patients at an increased risk for harm. This discussion recognises that patient autonomy and freedom of choice are key principles to be protected within this field, yet emphasis should also be placed on the proper regulation of expert practitioners and on the need for safe medical devices. The regulation of non-surgical cosmetic interventions holds substantial value for societal good, with an increase in safety, efficacy, accountability, and ultimately, patient well-being.

Keywords: Non-surgical interventions, Regulatory, European Union, United Kingdom, Patient safety, Medical device

Introduction

Augmenting the human physique is a concept that has existed across many cultures for thousands of years. As society and medicine have changed and evolved over this time period, the manner in which augmentation is conducted has also developed and grown[1]. In modern society, physical alteration can generally be described within the medical field as being cosmetic-orientated medicine[2]. This field can subsequently be split into surgical-based interventions and non-surgical-based interventions[2]. For the scope of this discussion, an in-depth look will be applied to non-surgical-based interventions and their regulation within the European Union (EU), as well as the United Kingdom (UK). In doing so, this discussion aims not only to identify what consists of non-surgical interventions and their subsequent current regulation, but to also highlight the implications of altering these existing regulatory laws.

Defining Non-Surgical Interventions

In order to be able to discuss the regulatory components surrounding "non-surgical interventions", one must first clearly identify what constitutes these procedures. Inherent to the definition itself, all approaches involving surgical components are excluded from this field. This leaves a wide variety of other procedures, with varying complexity and corresponding risk, excluded and unregulated. As highlighted by Mikhail et al. in 2019, non-surgical cosmetic procedures (NSCPs) refer to interventions that "are, broadly, used in order to enhance cosmetic appearance and mask signs of ageing, by altering volume and contours and by changing the quality of the skin."[3]

Mikhail et al. continues by stating that these types of procedures range from injections of dermal fillers or botulinum toxin, to hair transplantation and skin rejuvenation treatments[3]. The way these types of treatments are administered is relative to the regulations that are enforced in the country of practice. Currently, in the UK, NSCPs can be performed in a variety of environments, ranging from outpatient medical clinics to dentistry offices, "medical spas," and beauty salons. The latter settings are of particular concern due to the dramatic variation in training that practitioners have. As highlighted by Kamouna et al. in 2015, there have been direct consequences of patients having unnecessary side effects due to untrained individuals administering dermal fillers[4]. Exacerbating this problem is the escalating trend towards beauty salons providing this type of treatment. As a result, more patients have been presenting to hospitals with infections, often due to a lack of proper technique of untrained practitioners[5]. The expansion in

Table 1. **Sample of the Existing Regulation of Non-Surgical Procedure and Complications in the United Kingdom. (Referenced from United Kingdom Department of Health, 2013[6])**

Non-Surgical Cosmetic Procedure	Product Regulations	Medical Practitioner Required for Administration?	Location of Administration	Risks and Complications
Botulinum Toxin	Prescription Required	No	No Requirement	Bleeding, unintended muscle weakness, eyelid droop, double vision, speech and breathing difficulties, asymmetry, infection, allergic reaction. Not to be used in pregnancy
Chemical Peel	General Product Safety Directive if sold directly to end user	No	No Requirement	Burns, infection, scarring, changes in pigmentation, alteration of skin texture, persistent redness, asymmetry
Dermal Filler	Non-regulated unless self-described as a medical device (most do not)	No	No Requirement	Infection, scarring, persistent inflammatory response, thickening, pain, infection, asymmetry, tissue loss, poor aesthetic outcome, visual disturbance, blindness
Laser Treatment	Equipment is classified as a medical device	No	No Requirement	Burns, infection, changes in pigmentation, scarring, asymmetry, visual disturbance, blindness.
Intense Pulsed Light	Not considered a medical device	No	No Requirement	Burns, infection, changes in pigmentation, scarring

the number of patients receiving these procedures can be highlighted by the fact that NSCPs are a growing field, accounting for 9 out of 10 of all cosmetic procedures, and is estimated to be worth more than £3 billion in the UK alone[3]. With this lucrative field being responsible for over 75% of the market value in cosmetic interventions, it provides ample motivation for many different types of practitioners to become involved[6]. This is where the concept of regulation of the industry particularly comes into focus, and is directly linked to the risks that are associated with such procedures. Malpractice can result in complications that include poor injection technique, idiosyncratic immunological response, maiming, or other life-altering side effects[7,8] (**Table 1**).

Current Regulations in the European Union and the United Kingdom

Ensuring patient safety and practitioner expertise is firmly embedded into the concept of regulation within the medical field. As such, countries have various forms of medical legislation ranging from drug access regulations to requisites for conducting surgery[9]. Inconsistencies are apparent when contrasting the EU and the UK's regulation of non-surgical cosmetic intervention.

When examining the regulations surrounding devices that are used in NSCPs, the issue of the UK being untethered to European Union legislation has a profound impact. The UK has little to no quality control or safety requirements for its cosmetic products such as dermal fillers. As a result, individuals can easily order what may be a counterfeit or unsafe product through accessible retailers such as Amazon or Google[10]. One exception does exist; botulinum toxin is classified as a "prescription only medical device" due to its innate harmful characteristics. Despite this classification, it has been shown that non-medical practitioners are able to find a legal loophole in acquiring the toxin, with the only legal requirement being for a medical practitioner to prescribe the toxin to the patients at the clinic[11]. Unfortunately, this does not necessarily mean that the patient must attend a consultation with the doctor, but rather the toxin can be prescribed by proxy, and subsequently be administered by a non-medical practitioner. This practice is of much debate in its legality and ethics[11]. Furthermore, the application of these products is not regulated within the UK, and as such, there is no requirement for individuals to meet regulatory standards to administer substances such as botulinum toxin or dermal fillers[6].

In contrast, the member states of the EU fall under the European Medical Device Regulation which officially came into effect on May 26, 2021[12]. Crucial to being a regulation instead of its predecessor, the Medical Device Directive, these laws are directly applicable at a national level rather than having to go through subsequent country-level legislation[13]. Under this regulation, collagen implants, dermal fillers, skin resurfacing equipment, and laser hair removal equipment are all classified as medical devices. The aim is for NSCPs and products to meet the CE Mark Certification for safety, comply with quality management competencies according to EN ISO 13485:2016 standards, and to have an EU company registration if the company is located out of the EU[13]. Furthermore, the EU regulation surrounding the application and conduction of NSCPs is highly regulated, with the procedures being restricted to highly trained individuals with a medical license[14,15].

Advocacy for Change

When considering the differences between the legislation in the UK and the EU, one key issue that should be recognised is the primary motivation for the legislation. Modern medical practice emphasises the need to avoid paternalism and instead aims to promote patient autonomy and decision-making[16]. Therefore, it is imperative that the concept of medical regulation stems from an obligation to beneficence. The regulations in the EU are directed at this in two ways. First by ensuring the safety and quality of the products through the incorporation of CE marking, and second through restricting their usage to individuals with a medical license[14].

In direct comparison, the lack of regulation in the UK appears to subject a greater proportion of risk to patients. However, this does not indicate that individuals in the UK are content with the present level of regulation. A 2013 report, signed by former Medical Director of the UK National Health Service, Bruce Keogh, directly highlighted the vulnerabilities in the existing regulations surrounding non-surgical cosmetic interventions in the UK[6]. This report was specifically commissioned by the Department of Health in response to serious health

concerns regarding other cosmetic interventions[17]. To highlight the level of concern generated by the findings of this report, Keogh stated that, "All devices, whether they are solid or liquid, that are implanted into humans and stay there should be covered by the Medical Devices Regulations."[17] This was further elaborated on by the response of the health minister at the time, Dan Poulter, who stated, "The independent panel has made some far-reaching recommendations, the principles of which I agree with entirely. We will consider the report carefully and respond in detail in the summer."[17]

Yet, as time has gone on, the effects of the report findings have faced difficulty in their actual implementation, regardless of further support from the medical community in the need for new regulation. In an article published in 2015, Dr. Tamara Griffiths, a member of the British Association of Dermatologists, called again on the government for the establishment of a mandatory register for practitioners who conduct NSCPs[18]. She went on to elaborate on the rationale for having a mandatory register, stating that the lack of regulation leaves patients vulnerable to having to make medical decisions without being necessarily properly informed, and that it does not hold practitioners accountable for their actions[18]. However, the lack of substantial changes in legislation and regulation does not indicate a complete lack of action on behalf of the UK regulators. As highlighted in 2019, the publication of guidance from the Joint Council for Cosmetic Practitioners for non-surgical cosmetic treatments was described by Dr. Caroline Mills of the British Association for Oral and Maxillofacial Surgeons, as a step in the right direction[15]. Yet again though, Dr. Mills indicated that the guidelines did not go far enough, specifically stating that, "In the EU practitioners have to have a medical license to inject fillers, and we need similar regulation in the UK."[15] The lack of this regulation puts patients at serious medical complications argues Dr. Mills, with risks of severe allergic reactions or vascular occlusion being among the potential side effects[15].

Conclusion

Ultimately, when looking at the state of regulations in the UK in comparison to the EU it does not appear to be a matter of one region simply taking a different approach into the regulation of NSCPs, but rather a lack of action on the part of regulators in the UK. Furthermore, to simply state that it is a lack of attention to the issue that fuels this would be false, as evidenced by the commission of the 2013 Keogh report and the alteration in guidelines from the Joint Council for Cosmetic Practitioners in 2019. Additionally, it is rather easy to state that a country should simply adopt the regulations of another, but again this would be an overly simplistic statement to make—as much as one set of regulations may appear superior to another for various reasons, they still need to be adapted and implemented in a sustainable manner specific to the country. As such, it would likely be more effective for the UK to alter their regulations in a manner similar to that seen in the EU, or perhaps in a way that could be implemented more effectively than what presently exists.

Finally, self-identity and self-worth have a direct impact on an individual's mental health. How one goes about improving or achieving that is ultimately up to that individual, and as such, the freedom of choice to modify their body can be articulated as their right. However, it is arguably the responsibility of medical professionals and legislators to ensure that in providing these interventions they mitigate as much risk as possible. Through regulations that emphasise safety, accountability, ethical conduct, and informed consent, regulatory bodies not only help improve the implementation of non-surgical cosmetic interventions in Europe, but set standards that could be replicated across the globe. ◄

Declarations

The author declares no conflicts of interest.

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SYSTEMATIC REVIEW

The Pathogenesis, Risk Factors, and Comorbidities Associated with Hidradenitis Suppurativa: A Systematic Review

Róisín Guihen[a]*, Jack Latvis[a], Honora MacNamara[a], Emma Quinn[a], Sarah Hennessy[a], Cian Meagher[a], Julie MacMahon[a,b]

[a] *School of Medicine, Trinity College Dublin, University of Dublin, Ireland*
[b] *Department of Dermatology, Tallaght University Hospital, Tallaght, Dublin 24, Ireland*
**Corresponding author: GuihenR@tcd.ie*

Abstract

Introduction: Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease primarily affecting the apocrine gland-rich areas of the body. It presents with painful nodules, abscesses, sinus tracts, and scarring.

Methods: A literature review of hidradenitis suppurativa was conducted by systematically searching relevant databases with a focus on the pathogenesis and risk factors associated with the disease.

Results: Evidence relating to pathogenesis and HS thus far supports an inflammatory component with dysregulation of the innate and adaptive immune system. However, research is ongoing in this area and many questions remain unanswered. The risk factors that have been most consistently associated with HS to date include high weight/obesity, smoking, and female sex. Comorbidities in patients with HS encompass metabolic, endocrine, psychiatric, and inflammatory diseases.

Conclusion: Further research is warranted to enable clinicians with the knowledge necessary to manage patients presenting with HS and to deliver patients the disease-modifying treatment and care that they require. Several practical points may be discerned from research regarding risk factors and diseases associated with HS. These include raising the index of suspicion for certain physical diseases and mental conditions in patients with HS and lowering the biopsy threshold for certain malignancies.

Keywords: Hidradenitis suppurativa, Acne inversa, Systematic review

Introduction

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease primarily affecting the apocrine gland-rich areas of the body. It presents with painful nodules, abscesses, sinus tracts, and scarring[1]. It is a destructive, deforming disease, and has a significant impact on patient quality of life. The first clinician to describe HS was Velpeau in the year 1839[2]. However, it has only been acknowledged as a separate disease entity in recent years, as previously, it was thought of as a variation of acne vulgaris[3]. Much contemporary research has therefore been conducted on the disorder revealing important insights into its pathogenesis and risk factors. HS is now known to be a unique multifactorial disease whose pathogenesis involves an intricate interplay between genes and environmental factors[2]. This literature review aims to ascertain what research has revealed regarding the pathogenesis of HS, the risk factors, and diseases associated with the disease. In doing so, the authors intend to provide an up-to-date understanding of HS to assist in the clinical diagnosis, screening, and management of patients with this challenging disease.

Table 1. **Oxford Levels of Evidence**

Level	Type of Study
Ia	Systematic Review (SR)/Meta-analysis (MA)
Ib	Individual Randomised Control Trial
IIa	SR/MA of Cohort Studies
IIb	Individual Cohort Study
IIIa	SR/MA of Case Control Studies
IIIb	Individual Case Studies
IV	Case Series
V	Expert Opinion

Methods

Review of Papers Relating to Pathogenesis of HS

Ovid, PubMed, Web of Science, and the Cochrane Library were systematically searched using the keywords: "*hidradenitis suppurativa*" or "*acne inversa*". All papers

that were relevant to the review were included and any duplicates were removed. A total of 40 articles were identified. Only articles with the level of evidence IIIb according to the Oxford Centre of Evidence-Based Medicine **(Table 1)** or superior have been included, any levels of evidence below this were deemed not to have an adequate level of objectivity. As HS was only considered a disease entity in itself in the 1990s, articles from the period of 1990–2018 were selected for inclusion. Other reasons for exclusion were that the text was not available in full, or that it was written in a non-English language.

Of all papers screened using these parameters, 19 were excluded, leaving 21 which were fully assessed for inclusion. 1 paper was subsequently excluded. Thus, 20 studies in total were selected for inclusion in this review **(Figure 1)**.

Review of Papers Relating to Risk Factors of HS

The review of literature pertaining to the risk factors and diseases associated with HS was conducted as above for papers relating to the pathogenesis of HS.

A total of 48 articles were collected. Of all papers screened using the parameters defined above, 10 were excluded. The 38 remaining articles were fully assessed for inclusion, with none of these being subsequently excluded. In total, 38 studies were used for the data in this review **(Figure 2)**.

Results and Discussion

Pathogenesis and Mechanism of HS

Our search results for pathogenesis yielded 20 papers in total: 9 case reports, 6 case-control studies, and 5 cohort studies, as summarised in **Table 2**.

The papers encompassed a wide array of data pertaining to various theories and hypotheses regarding the pathogenesis of HS. Although the pathogenesis of HS is still not well-understood, the data maintains that there appears to be a dysregulation of the adaptive and innate immune systems. For instance, one cohort study identified an upregulation in certain inflammatory cell types in HS including IFN-γ and TNF-β/LT-α[23].

A genetic link conferring a predisposition for the development of HS in certain forms of the disease has also been identified. A case report in 2017 stated that HS can be caused by a mutation in γ secretase which is responsible for the Notch signalling pathway[9]. This theory is also supported by a cohort study from 2012 in which the γ-secretase genes NCSTN, PSENEN, and PSEN1 were shown to contribute to rare forms of HS[21]. The Notch pathway is important for maintaining the inner and outer root sheath of the hair follicles. When there is deficient Notch signalling it results in conversion of hair follicles to keratin-enriched epidermal cysts. This can result in a Toll-Like Receptor (TLR)-mediated innate immunity.

A case report from 2017 defined HS as an inflammatory disorder of the follicular epithelium, however a secondary bacterial infection can often occur[9]. A cohort study published in 2018 suggested that HS is due to an exaggerated inflammatory response which may be driven by dysbiotic commensal bacteria and/or biofilms[20].

While a lot of attention has been given to the cellular and molecular biology of the host tissues affected by HS, less attention has been given to the bacteria involved and the potential involvement of the cutaneous microbiome. A case report conducted in 2012 suggested that HS should be considered as part of bacterial biofilm-based disorders due to the clinical features of HS couple with the supporting bacterial communities[12]. A case-control study published in 2017 investigated whether the cutaneous microbiome of 30

Figure 1. **Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) Chart for Papers Relating to Pathogenesis of HS**

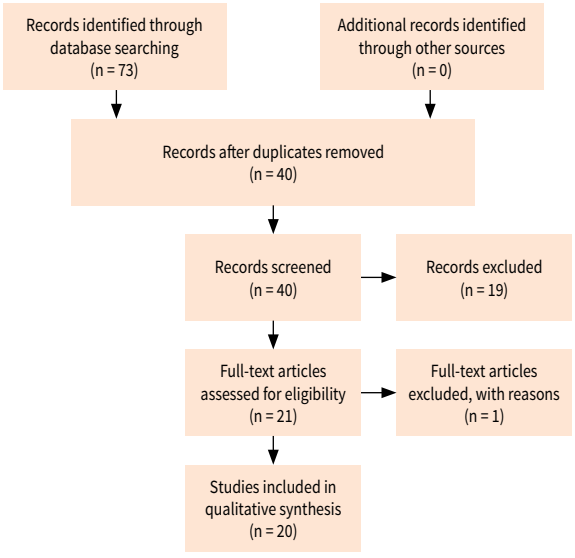
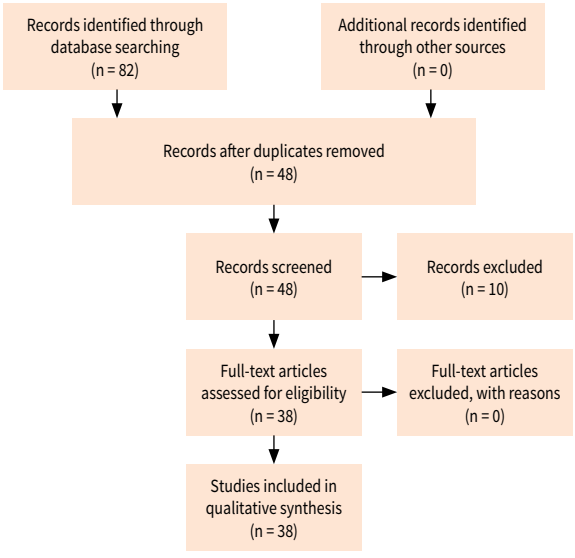


Figure 2. **PRISMA Chart for Papers Relating to Risk Factors of HS**



HS patients differed from that in 24 healthy controls[15]. They identified five microbiome types and their resulting data suggested that a dysbiotic microbiome may have a role in the pathogenesis of HS. The use of next-generation sequencing analysis provided a previously unreported description of the microbiome in HS as previous studies had relied on culture-based methods, which often suggested commensal bacteria[22]. Previous studies have also been restricted to lesional skin while this study included both non-lesional skin of HS patients and healthy controls.

A case-control study conducted in 2017 investigated the global DNA methylation and hydroxymethylation status in lesional and perilesional HS skin compared to healthy controls. To date there have been no previous reports on the imbalance between methylation and demethylation concerning patients with HS. While their results showed no difference in global DNA methylation, they did find an imbalance in DNA hydroxymethylation suggesting that it may play a role in the pathogenesis of HS and hence may pose as a future therapeutic target[13].

Risk Factors and Associated Disease

This section of the review encompassed 38 papers in total: 8 case reports, 11 case-control studies, and 19 cohort studies relating to the risk factors and diseases associated with HS. These are listed in **Table 3**.

Although this provided a wide scope of data pertaining to the factors with HS, it must be noted that some studies did not provide as much detail as to which risk factors and comorbidities were accounted for in their sample as others. Therefore, several associated factors may appear rarer than their actual clinical frequency due to their omission from select studies' interest criteria. Several other associated factors may have seen an antithetical rise in their apparent clinical frequencies due to a trend of inflated interest in those factors at some time. Non-modifiable risk factors that have been consistently identified include female sex and a positive family history. Those factors and co-morbidities, which appear to have the most bearing on clinical practice, will be henceforth discussed. These include weight, inflammatory bowel conditions, cardiovascular disease, psychiatric disorders, and malignancies.

Associated factors pertaining to increased weight, namely obesity, overweight, and high BMI, were among the most commonly observed, collectively appearing in 21 of the papers reviewed. Factors in the arena of smoking were also found to be highly frequent fixtures, with 23 papers marking the activity a significant factor in their investigations. Factors relating to malignancy were also elucidated as significant in a selection of studies. Most notable among these factors were those of squamous cell carcinoma (SCC) (4 studies), hepatic carcinoma (2 studies), and haematological malignancies (2 studies). Associated factors regarding inflammatory bowel disease (IBD) were highlighted in 4 studies, chief among the factors being Crohn's disease, which was discussed in all 4 of the same papers. Furthermore, Egeberg, Jemec, et al. discovered a significantly increased risk of new-onset Crohn's disease and new-onset ulcerative colitis in patients with HS, implying that these IBDs are more likely to be comorbidities of HS than risk factors for the development of HS[49].

A cohort study performed by Egeberg, Gislason, et al. determined there to be a significantly increased risk of cardiovascular-associated death in patients with HS, pointing to increased risks of myocardial infarction and ischaemic stroke as pertinent comorbidities of the disease[55]. Hypertension was found to be another cardiovascular associated factor, with 7 studies reporting its significance, as were subclinical atherosclerosis[36,37], greater carotid intima-media thickness[37], and carotid plaques[37].

Psychiatric disorders were found to be significantly associated factors in 5 papers. Depression was marked as a significant factor in 3 cohort studies, while

Table 2. **Search Results for the Pathogenesis and Mechanism of HS**

Pathogenesis Factors	Number of Papers				Reference(s)
	Total	a) Case Reports	b) Case Control Studies	c) Cohort Studies	
Enhanced expression of tumour necrosis factor (TNF)-α	7	6	0	1	a) 4, 5, 6, 7, 8, 9 c) 20
Enhanced expression of interleukin (IL)-1β	2	1	0	1	a) 4 c) 18
Enhanced expression of IL-17	4	2	0	2	a) 4,5 c) 18, 20
Loss of function of γ-secretase multiprotein complex which regulates the Notch signalling pathway	3	2	0	1	a) 6, 9 c) 21
IL-12/Th1 pathway elevation	1	1	0	0	a) 5
Deficiency of IL-22 and IL-20 in HS lesions	1	1	0	0	a) 5
Abnormal expression of innate immunity markers (toll-like receptors 2, 3, 4, 7, and 9; intercellular adhesion molecule 1; interleukin [IL] 6 and 10; tumour necrosis factor; α-melanocyte stimulating hormone; transforming growth factor β; β-defensin 2)	9	6	1	2	a) 4, 5, 6, 7, 8 b) 17 c) 18, 20
Blockage of IL-2	1	1	0	0	a) 10
Prosthesis-related friction	1	1	0	0	a) 11
Bacteria biofilm	6	2	3	1	a) 7, 12, b) 15, 16, 19 c) 22
Interferon-gamma (IFN-γ) elevated in the HS	1	0	0	1	c) 23
Higher levels of tumour necrosis factor-β (TNF-β)	1	0	0	1	c) 23
Increase in serum C5a	1	0	0	1	c) 20
Reduction of components in the proximal part of the complement pathway (C3, C4, and iC3b)	1	0	0	1	c) 20
Imbalances in DNA hydroxylation	1	0	0	1	b) 13
Role of anti-cytokine autoantibodies (c-aAbs)	1	0	0	1	b) 14

Table 3. **Search Results for Risk Factors and Diseases Associated with HS**

Associated Factor(s)	Number of Papers: Total	a) Case Reports	b) Case Control Studies	c) Cohort Studies	Reference
Obesity/Overweight/High BMI (Body Mass Index)	21	2	10	9	a) 24, 25 b) 22, 32, 34, 35, 36, 37, 38, 39, 40, 42 c) 33, 43, 44, 45, 48, 50, 51, 52, 56
Increased waist circumference	4	0	4	0	b) 32, 33, 36, 39
Smoking	23	4	6	13	a) 22, 24, 25, 28 b) 32, 35, 37, 38, 40, 58 c) 33, 43, 44, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57
High pack year history	4	2	2	0	a) 24, 25 b) 32, 40
Current smoker	5	0	3	2	b) 35, 37, 40 c) 43, 44
Former smoker	4	0	2	2	b) 32, 35 c) 43, 44
Squamous cell carcinoma	4	3	2	1	a) 25, 26, 29 c) 53
Haematological malignancy	2	1	0	1	a) 27 c) 51
Acute leukaemia	1	0	0	1	c) 51
Hepatic carcinoma	2	1	0	1	a) 31 c) 60
Primary liver cancer	1	0	0	1	c) 60
Diffuse malignant peritoneal mesothelioma	1	1	0	0	a) 29
Non-melanoma skin cancer	1	0	0	1	c) 60
Buccal cancer	1	0	0	1	c) 60
Lymphoma	1	0	0	1	c) 51
Inflammatory bowel disease	4	0	1	3	b) 35 c) 49, 56, 57
Crohn's disease	4	0	1	3	b) 35 c) 49, 56, 57
Ileocolonic and/or perianal Crohn's sisease	1	0	1	0	b) 35
New-onset Crohn's disease	1	0	0	1	c) 49
New-onset ulcerative colitis	1	0	0	1	c) 49
Positive family history of HS	5	1	2	2	a) 24 b) 38, 58 c) 33, 44
Hypertension	7	2	1	4	a) 25, 31 b) 36 c) 43, 51, 56, 58
Renal dysfunction	2	1	0	1	a) 30 c) 43
End stage renal disease	1	1	0	0	a) 30
Diabetes mellitus	11	1	1	9	a) 31 b) 36 c) 43, 44, 47, 48, 49, 51, 56, 60
Gout	1	1	0	0	a) 31
Metabolic syndrome	3	0	3	0	b) 32, 34, 36
High triglycerides	3	0	3	0	b) 32, 36, 39
Low high-density lipoprotein cholesterol (HDL-C)	4	0	4	0	b) 32, 36, 38, 39
High very low- density lipoprotein (VLDL-C)	1	0	1	0	b) 32
High serum insulin	2	0	2	0	b) 32, 42
High C-reactive protein (CRP)	3	0	2	1	b) 32, 42 c) 56
High high-sensitivity C-reactive protein (hs-CRP)	3	0	3	0	b) 32, 36, 37
High homocysteine	1	0	1	0	b) 32
High uric acid	1	0	1	0	b) 32
Elevated erythrocyte sedimentation rate	3	0	3	0	b) 32, 36, 37
High homeostatic model of insulin resistance	2	0	2	0	b) 32, 34
Serum visfatin levels	1	0	1	0	b) 32
Increased systolic and diastolic blood pressure	2	0	2	0	b) 34, 39
High serum glucose levels	3	0	3	0	b) 34, 37, 39
High NOD-like receptor levels	1	0	1	0	b) 36
Subclinical atherosclerosis	2	0	2	0	b) 36, 37
Low total cholesterol	1	0	1	0	b) 37
Greater carotid intima-media thickness	1	0	1	0	b) 37
Carotid plaques	1	0	1	0	b) 37
Female sex	15	0	2	13	b) 38, 42 c) 43, 44, 45, 46, 47, 48, 49, 50, 51, 55, 56, 57, 58
Genital HPV (human papillomavirus) infection	1	0	1	0	b) 41
Increase in neutrophilocytes	2	0	1	1	b) 32 c) 56
Increase in lymphocytes	1	0	1	0	b) 42
Increase in leucocytes	2	0	1	1	b) 42 c) 56
Acne	3	0	0	3	c) 44, 45, 58
Black race	2	0	0	2	c) 45, 50
White race	3	0	0	3	c) 48, 50, 54
Alcohol abuse	3	0	0	3	c) 47, 52, 60
Psychiatric disorder	5	1	0	4	a) 31 c) 47, 50, 51, 56
Schizophrenia	1	1	0	0	a) 31
Depression	3	0	0	3	c) 47, 50, 51
Anxiety	1	0	0	1	c) 47
Antidepressant drug use	1	0	0	1	c) 47
Anxiolytic drug use	1	0	0	1	c) 47
Hospitalisation due to anxiety	1	0	0	1	c) 47
Hospitalisation due to depression	1	0	0	1	c) 47
Increased risk of completed suicide	1	0	0	1	c) 47
Drug addiction	1	0	0	1	c) 55
Age in 3rd or 4th decade of life	1	0	0	1	c) 48
Chronic pain	2	0	0	1	c) 50, 59
Iron-deficiency anaemia	1	0	0	1	c) 51
Pilonidal disease	1	0	0	1	c) 51
Liver disease	2	0	0	1	c) 51, 60
Low socioeconomic status	2	0	0	1	c) 52, 55
Increased risk of myocardial infarction	1	0	0	1	c) 55
Increased risk of ischaemic stroke	1	0	0	1	c) 55
Increased risk of cardiovascular-associated death	1	0	0	1	c) 55
Increased risk of major adverse cardiac events	1	0	0	1	c) 55
Increased risk of all-cause mortality	1	0	0	1	c) 55
Increased risk of adverse cardiovascular outcomes	1	0	0	1	c) 55
Psoriasis	1	0	0	1	c) 56
Polycystic ovary syndrome	1	0	0	1	c) 56
Hypothyroidism	1	0	0	1	c) 56
Atopic dermatitis	1	0	0	1	c) 56
Post-colectomy pouchitis	1	0	0	1	c) 57
Impairment of self-perception	1	0	0	1	c) 59
Impairment of daily living activities	1	0	0	1	c) 59
Impairment of mood state	1	0	0	1	c) 59
Physical discomfort	1	0	0	1	c) 59

schizophrenia, anxiety and drug addiction were factors of similar magnitude in one paper each. It is worthy to note that potential HS-associated factors relating to psychiatric disorders are not explored beyond these 5 papers in either a confirmatory or disproving sense among the remainder of the studies compiled here, pointing to a dearth of interest in furthering research that is inclusive of this realm. The importance of scrutinising the psychiatric comorbidities and risk factors of HS is attested to by the recent work of Thorlacius et al., who discovered a significantly increased risk of completed suicide in patients with HS[47]. Thus, probing the depths of HS's psychiatric associations could prove lifesaving or, at the very least, a benefit to the wellbeing and treatment of patients afflicted by the disease.

Conclusion

Summary of Results

In conclusion, the pathogenesis of HS is yet disputed. Evidence thus far supports an inflammatory component with dysregulation of the innate and adaptive immune systems. Research is ongoing however and with the emergence of modern, more promising research methods, a more concrete characterisation of the pathogenesis of HS is likely to emerge. This in turn will help in the quest to identify and develop better treatment options for afflicted patients.

The risk factors that have been most consistently associated with HS in the research to date include high weight/obesity, smoking, and female sex. Comorbidities in patients with HS encompass metabolic, endocrine, psychiatric, and inflammatory diseases. Though research indicates that the diseases/factors discussed earlier in this review are associated with HS, no common pathogenetic background has yet been determined. Moreover, whether these associated diseases and risk factors are a cause of—or are more often caused by—HS is yet to be conclusively discerned.

Relevance to Clinical Practice

Many questions remain unanswered in the search to identify the pathogenesis and mechanism of HS and provide an understanding of its link to specific risk factors and diseases. Further research is thus warranted to provide clinicians with the knowledge necessary to manage patients presenting with this debilitating disease and to the disease-modifying treatment and care they require.

With regards to empirical work conducted on the risk factors and diseases associated with HS, several points for the practicing clinician may be extracted. Clinicians should consider that HS patients may have ≥1 undiagnosed components of metabolic, endocrine, psychiatric or inflammatory disorders, despite their youth, and initiate appropriate targeted screening. The association between depression, mood disorders and HS should be acknowledged in clinical practice and sought for in the patient presenting with a HS picture. Finally, clinicians should raise their index of suspicion for SCC malignancy and lower their biopsy threshold in HS patients to prevent or minimise SCC metastasis. ◄

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Declarations

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SYSTEMATIC REVIEW

In Patients Admitted to ICU with SARS-CoV-2 Infection, is Dexamethasone Superior to Standard Care in Improving Mortality? A Systematic Review of Evidence to Date

Laith Al Azawi[a], Conor Farrell[a], Lauren Hayes[a]*, Liam Mariga[a], Imad Mirza[a], Mariam Salem[a]

[a] *School of Medicine, Trinity College Dublin, University of Dublin, Ireland*
* *Corresponding author: Hayes14@tcd.ie*

Abstract

Introduction: Dexamethasone is a potent broad-spectrum corticosteroid that decreases the transcription of pro-inflammatory cytokines, whilst simultaneously increasing the transcription of anti-inflammatory cytokines. The cytokine storm that is central to the pathogenesis of acute respiratory distress syndrome (ARDS) and multi-organ failure is seen in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) related deaths. The objective of the study was to systematically review the use of dexamethasone for COVID-19 in adult ICU patients and to ascertain if there was a survival benefit compared to standard care (SC) alone.

Methods: A literature search of two databases, EMBASE and PubMed, was conducted using the terms "COVID-19", "Dexamethasone", and "ICU". The search was limited to studies published in the English language. The PRISMA guidelines were used to guide our search methodology.

Results: The database search identified 59 articles. Of these, two duplicates were discarded, and 57 studies were screened. 54 of these publications were deemed irrelevant based on the inclusion and exclusion criteria. Three were forwarded for full text review and met inclusion and exclusion criteria on full-text review. All three were deemed eligible. The selected studies consisted of two randomised controlled trials (RCTs) and one case series report. The results from the three papers were unanimous in their conclusion that dexamethasone was superior to SC in the treatment of patients admitted to ICU with SARS-CoV-2. There was also a shorter duration of hospitalisation seen in the patient group treated with dexamethasone.

Conclusion: Our systematic review found that dexamethasone was superior to SC in patients admitted to the ICU with SARS-CoV-2 infection. However, administration of dexamethasone to patients not on respiratory support resulted in a higher incidence of death, compared to SC.

Keywords: COVID-19, Dexamethasone, Intensive care unit

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly transmissible airborne virus that has led to the COVID-19 global pandemic due to its rapid spread via human-to-human transmission. The main pathophysiological findings of COVID-19 include diffuse alveolar damage, acute pneumonia with opacity clearly seen on a chest radiographic image and infiltration of inflammatory cells. The clinical course of this disease is highly heterogeneous, ranging from an asymptomatic presentation to varying degrees of hypoxia, acute respiratory distress syndrome (ARDS), and ultimately death in a considerable number with an infection fatality rate of 0.68% across populations[1]. The mortality rate is higher in those with comorbid conditions, namely diabetes, hypertension, heart failure, and obesity[2].

Most COVID-19 positive patients in the intensive care unit (ICU) require supplemental oxygen up to, and including, prolonged invasive mechanical ventilation[3]. This is due to persistent hypoxaemia and a reduced ventilation-perfusion ratio secondary to alveolar damage. Persistent hypoxaemia can induce a cascade of multi-organ failure if the precipitating inflammation is not managed[4]. Dexamethasone is a potent broad-spectrum corticosteroid that works by decreasing the gene transcription of pro-inflammatory cytokines while increasing the transcription of anti-inflammatory cytokines, therefore reducing the likelihood of the cytokine storm syndrome that can lead to ARDS and multi-organ failure seen in COVID-19 associated deaths[5]. The RECOVERY trial selected dexamethasone as the corticosteroid of choice that reduced mortality in SARS-CoV-2 patients with ventilatory support when administered for 10 days[6]. Therefore, this study will focus on dexamethasone, specifically in severe cases of COVID-19 seen in ICU patients.

The objective of this study is to perform a systematic review of the published literature to date to ascertain if dexamethasone provides a survival benefit as compared to standard care (SC) alone for patients with SARS-CoV-2 infection in the ICU setting. SC for SARS-CoV-2 treatment involves a combination of antivirals and immune modulators[1]. Antivirals commonly used include lopinavir-ritonavir, ribavirin, and hydroxychloroquine. Immune modulators included tocilizumab and convalescent plasma. Other clinical pharmacological interventions included a variety of antibiotics (meropenem, piperacillin/tazobactam, doxycycline, linezolid, and azithromycin) and anticoagulants, likely used as part of venous thromboembolism prophylaxis.

There have been multiple trials carried out over the last year examining the effect of dexamethasone on these patients. Our objective is to compile these findings and review whether the drug is effective and whether there have been any complications or specific patient groups where the drug has had adverse effects.

Methods

This study was performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) protocol[7] as shown in **Figure 1**.

Information Sources and Search Strategy

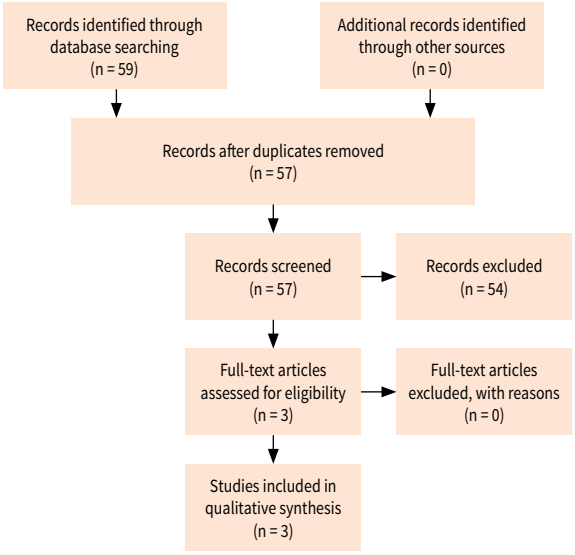
Publications from both EMBASE and PubMed databases were screened. The search parameters used included: "COVID-19", "Dexamethasone" and "ICU" with synonyms included. The chosen population were adult ICU patients with COVID-19 and the chosen intervention was exclusively dexamethasone.

Eligibility Criteria

Exclusion criteria included abstract only papers, non-English papers, and animal studies. The inclusion criteria were specific to ensure a representative sample. They were English language only, full text availability and human trials only. The type of papers included were randomised clinical trials (RCTs), systematic reviews, case studies, cohort analysis studies, meta-analyses, and multicentre and observational studies. The timeline for studies included was from December 2019, when COVID-19 just emerged, to the date the search took place.

The combined database search results were then reviewed. The remaining titles and abstracts were independently screened by a reviewer without consideration for the results. During this process, any article that did not meet our inclusion criteria was excluded. Deduplication was performed using "Covidence", a software management platform for systematic reviews[8].

Figure 1. **PRISMA Flow Diagram**



Results

The database searches yielded 57 results following deduplication. After review of titles and abstracts by a single reviewer, three studies remained to be included in the final study. These were then analysed, and data was extracted, compared, and compiled (**Table 1** & **Table 2**). These were: an RCT by Horby et al. in which the baseline was all hospitalised patients with confirmed SARS-CoV-2, including both ventilated and non-ventilated patients[9]; an RCT by Tomazini et al. in which all patients, 63% of whom were male, were receiving mechanical ventilation after a laboratory diagnosis of SARS-CoV-2 within 48 hours of meeting the criteria for moderate to severe ARDS according to the Berlin definition[10,11], and a case series report by Hassan et al. which included five patients that had acute lung injury (ALI) scores ranging from 1.25–3[12].

Of the three papers analysed, two were RCTs and one was a case series. As per the Oxford 2011 Levels of Evidence, the two clinical trials were designated as level 1b evidence, and the case series was designated as level 4 evidence. The first RCT was carried out in the United Kingdom[9] and the second was from Brazil[10], while the case series originated from Bahrain[12]. The average age in the RCTs was 65.8 years while it was 56.6 in the case series. Patients were predominantly male in the RCTs while all patients in the case series were female. The inclusion criteria for the RCTs varied but both included patients diagnosed with COVID-19 on invasive mechanical ventilation. Patient comorbidities were explicitly mentioned in one RCT[9] and in the case series[11], but not in the second RCT[10]. The dosage of dexamethasone used varied between the three papers (6mg–20mg) but were all administered once daily and intravenously. Similarly, SC varied greatly between the three papers and varied for each individual patient. In the paper by Horby et al. SC consisted of hydroxychloroquine, lopinavir-ritonavir, azithromycin, tocilizumab, and convalescent plasma[9]. In the paper by Tomazini et al. only one patient received lopinavir-ritonavir treatment, and the use of antibiotics and haemodynamic management varied between patients and were at the discretion of the ICU staff[10]. The SC in the paper by Hassan et al. also varied by patient: for

Table 1. **Summary of Papers Reviewed (RCTs)**

Title of Paper, Author, Year, Country of Origin	a) Sample Size (n) b) Mean Age c) % Male	Inclusion/ Eligibility	a) Oxygen/ Ventilation b) Median days since onset of symptoms/ hospitalization	Comorbidities	Dexamethasone (DM) Dose and Route of Administration	Comparator/Standard Care (SC)	Primary Outcome	Secondary Outcomes	Secondary Outcome Results
Dexamethasone (DM) in Hospitalized Patients with Covid-19 – Preliminary Report, Horby et al. 2020, United Kingdom[9]	a) n=6425 (DM=2104, SC=4321) b) 66.1 +/- 15.7 c) 64%	Hospitalised patients with suspected/ laboratory confirmed COVID-19 No age limit	a) 16% invasive mechanical ventilation/ extracorporeal membrane oxygenation, 60% oxygen only (with/ without non-invasive ventilation), 24% neither b) DM group: 8 days since onset of symptoms, 2 days since hospitalisation, SC group: 9 days since onset of symptoms, 2 days since hospitalization	Diabetes 24% Heart disease 27% Chronic lung disease 21%	Standard care + IV DM 6mg OD up to 10 days/ hospital discharge if sooner	Randomisation of patients to receive hydroxychloroquine, lopinavir-ritonavir, azithromycin, tocilizumab, convalescent plasma	All-cause mortality within 28 days of randomisation	Time until discharge from hospital Receipt of invasive mechanical ventilation Death (preceding 2 outcomes only apply if not receiving ventilation)	DM group shorter duration of hospitalization (median 12 days vs 13 days) and greater probability of discharge alive within 28 days (67.2% DM vs 63.5% SC) (RR 1.10) (95% CI 1.03-1.17) Patients who progressed to requirement of invasive mechanical ventilation/ subsequent death lower in DM group (25.6% vs 27.3%) (RR 0.92) (95% CI 0.84-1.01)
Effect of Dexamethasone on Days Alive and Ventilator-Free in Patients with Moderate or Severe Acute Respiratory Distress Syndrome and COVID-19: The CoDEX Randomised Clinical Trial, Tomazini et al. 2020, Brazil[10]	a) n=299 (DM=151, SC=148) b) 61+/- 14 c) 63%	Confirmed or suspected COVID-19 infection At least 18 years old Receiving mechanical ventilation within 48 hours of meeting criteria for moderate to severe ARDS (using Berlin Definition) with PaO2:FiO2 ratio of 200 or less	Mechanical ventilation was an inclusion criterion	Not explicitly stated	Standard care + IV DM 20mg OD for 5 days followed by IV DM 10mg for 5 days/ ICU discharge if sooner	1 patient received lopinavir-ritonavir treatment. Other therapeutic strategies such as tocilizumab and convalescent plasma were limited and not widely available 52 patients (35.1%) received at least 1 dose of corticosteroids, of whom 38 (73.1%) had other established clinical indications for corticosteroid use. The use of corticosteroids in 14 patients (9.4%) was considered a protocol deviation All clinical interventions, such as use of antibiotics, ventilatory strategy, laboratory testing, and hemodynamic management were left at the discretion of the ICU team for both groups	Ventilator-free days during the first 28 days, which was defined as being alive and free from mechanical ventilation	All-cause mortality at 28 days Clinical status of patients at day 15 using a 6-point ordinal scale (ranging from 1, not hospitalized to 6, death) ICU-free days during the first 28 days Mechanical ventilation duration at 28 days Sequential Organ Failure Assessment (SOFA) scores (range, 0-24, with higher scores indicating greater organ dysfunction) at 48 hours, 72 hours, and 7 days	There was no significant difference in the prespecified secondary outcomes of: all-cause mortality at 28 days (56.3% in the dexamethasone group vs 61.5% the standard care group; hazard ratio, 0.97; 95% CI, 0.72 to 1.31; P=.85); and ICU-free days during the first 28 days, (mean, 2.1; 95% CI, 1.0 to 4.5 days for the dexamethasone group vs mean, 2.0; 95% CI, 0.8 to 4.2 days for the standard care group; difference, 0.28; 95% CI, −0.49 to 1.02; P=.50) Mechanical ventilation duration at 28 days (12.5; 95% CI, 11.2 to 13.8 days for the dexamethasone group vs 13.9, 95% CI, 12.7 to 15.1 days for the standard care group; difference, −1.54; 95% CI, −3.24 to −0.12; P=.11) 6-point ordinal scale at 15 days (median, 5; IQR, 3-6 for the dexamethasone group vs median, 5; IQR, 5-6 for standard care group; odds ratio [OR], 0.66; 95% CI, 0.39 to 1.13; P=.07) At 7 days, patients in the dexamethasone group had a mean SOFA score of 6.1 (95% CI, 5.5-6.7) vs 7.5 (95% CI, 6.9-8.1) in the standard care group (difference, −1.16; 95% CI, −1.94 to −0.38; P=.004)

Abbreviations: RR = Respiratory Rate

Table 2. **Summary of Papers Reviewed (Case Report)**

Title of Paper, Author, Year, Country of Origin	Case	Age	Gender	Comorbidities	Date Diagnosed	ALI Score	Standard Care	Dexamethasone Commenced (Dose)	Labs
Dexamethasone in Severe COVID-19 Infection: A Case Series, Hassan et al. 2020, Bahrain[12]	1	38	F	Down syndrome	June 12	2.5	Lopinavir, Ritonavir, ribavirin, meropenem, LMWH, linezolid, doxycycline (June 12)	June 18 (IV 6mg OD)	C-reactive protein (CRP) declined from 227.6 to 17.5 mg/L; D-dimer (DD) from 21.55 to 4.94 µg/ml; lactate dehydrogenase (LDH) from 577 to 486 U/L; interleukin-6 (IL-6) from 15.2 to 11.39 pg/ml; and total white blood cell (WBC) count from 13.14 to 8.62 × 109/L
	2	44	F	Hypertension, Obesity	June 13	3	Lopinavir/ritonavir, ribavirin and interferon-β (June 13) Convalescent plasma (June 16)	June 18 (IV 6mg OD)	CRP declined from 69.4 to 14.4 mg/L; DD from 6.7 to 4.3 µg/ml; and IL-6 from 16.13 to 3.56 pg/ml.
	3	85	F	Hypertension, Hyperlipidaemia, Hypothyroidism	June 21	2.5	Lopinavir/ritonavir, interferon-β, linezolid, meropenem and enoxaparin (June 21) Convalescent plasma therapy x2, (June 26)	June 26 (IV 6mg OD)	CRP continued to increase from 31.2 to 276.8 mg/L; DD decreased from 14.3 to 3.16 µg/ml; LDH increased from 312 to 539 U/L; and WBC increased 12.1 to 14.3 × 109/L.
	4	45	F	None	June 19	3	Ribavirin, enoxaparin, piperacillin/tazobactam, doxycycline, convalescent plasma therapy x2	Not recorded (IV 6mg OD)	CRP declined from 152 to 32.9 mg/L; and LDH changed from 535 to 540 U/L
	5	71	F	Not explicitly stated	June 25	1.25	Lopinavir/ritonavir, interferon-β, ribavirin, piperacillin, doxycycline, and enoxaparin	Not recorded (IV 6mg OD)	CRP declined from 70.97 to 30.6mg/L

example, some patients received interferon therapy, and one patient received two courses of convalescent plasma therapy, which the other patients did not receive[12].

The three papers collectively showed that dexamethasone was superior to SC in the treatment of patients admitted to the ICU with SARS-CoV-2 infection. Horby et al. showed that the incidence of death was significantly lower in those on invasive mechanical ventilation (29.3% dexamethasone vs 41.4% SC, RR: 0.64, 95% CI: 0.51–0.81, Number Needed to Treat (NNT)=9), and was lower in patients on oxygen without invasive mechanical ventilation (23.3% dexamethasone vs 26.2% SC, RR: 0.82, 95% CI: 0.72–0.94, NNT=35)[9]. However, in this study, patients who were not on respiratory support, administration of dexamethasone resulted in a higher incidence of death than those that were given SC (17.8% dexamethasone vs 14% SC, RR: 1.19, 95% CI: 0.91–1.55, NNT=27). Overall mortality at 28 days among all patient groups was lower in those administered dexamethasone (22.9% vs 25.7% SC, RR: 0.83, 95% CI: 0.75–0.93, NNT=36) and there was a greater probability of being discharged alive within 28 days in the dexamethasone group (67.2% vs 63.5% SC, RR: 1.10, 95% CI: 1.03–1.17)[9].

Tomazini et al. showed that ventilator free days during the first 28 days was higher in those treated with dexamethasone (6.6 days [95% CI: 5.0–8.2 days] vs 4.0 days SC [95% CI: 2.9–5.4 days], difference 2.26, 95% C: 0.2–4.38)[10]. In this study, there was no significant difference in the prespecified secondary outcomes, including all-cause mortality at 28 days (56.3% dexamethasone vs 61.5% SC, RR: 0.97, 95% CI: 0.72–1.31).

The case series showed that dexamethasone had a possible protective effect in severe COVID-19 infections with significant improvement in laboratory markers including CRP, D-dimer, and IL-6[12]. In this study, there was also an observed general improvement in patient outcomes in the ICU.

Discussion

In this paper, three studies with a combined total of 6,729 patients from three different countries were reviewed. Upon analysis, all three studies supported the use of dexamethasone along with SC in patients requiring supplemental oxygen, rather than SC *alone*. The administration of dexamethasone reduced all-cause mortality at 28 days, increased ventilator free days, and improved laboratory markers of inflammation, namely CRP[9,10,12].

However, in spite of these promising results, it is important to note the difference in treatment outcomes in patients of particular groups. It is consistent across all three papers that dexamethasone is most effective in those invasively mechanically ventilated. This fact is particularly evident in Horby et al. where incidence of death in patients receiving invasive mechanical ventilation was 12.1% lower when dexamethasone was administered[9]. While substantial evidence can be drawn from these studies, showing that dexamethasone improves mortality in mechanically ventilated patients, little information is given about those requiring non-invasive ventilation or supplemental oxygen alone. For example, in the same study, limited information for patients who required oxygen but did not need mechanical ventilation was given. The results did suggest a reduction in the incidence of death within this cohort of patients (receiving oxygen without invasive mechanical ventilation) when treated with dexamethasone, however, the reduction is not as significant as in the invasive mechanically ventilated group[9]. It is difficult to determine possible reasons for this due to the limited information provided.

Information on the oxygen requirements or use of continuous positive airway pressure (CPAP) was not provided for the non-invasive ventilation group.

Another interesting finding was the detrimental effect of dexamethasone treatment on patients who did not require any respiratory support. Horby et al. clearly showed an increase in the incidence of death with the administration of dexamethasone to patients who were not receiving any respiratory support[9]. A possible explanation for this finding is that those who were mechanically ventilated were likely to have developed ARDS as a result of the immune system becoming hyper-responsive and causing a cytokine storm. In this study, the development and progression of ARDS could be a causative factor of death in this group[9]. However, the administration of dexamethasone, through the drug's anti-inflammatory effects, could possibly dampen down the immune system and subsequently reduce mortality. In contrast to this, those who were not mechanically ventilated would have been more likely to have a normal functioning immune system that was at least partially capable of clearing the virus. It was less likely for the immune system to be in a hyper-responsive state and therefore a lower likelihood of developing ARDS. An abnormal immune response would, as a result, not be as large of a threat to the patient's life in this group unlike those who were invasively mechanically ventilated. Therefore, administering dexamethasone would not be most suitable for this cohort of patients and could inadvertently increase mortality as the virus is permitted to replicate further.

Since the conclusion of our own research, various systematic reviews commenting on the efficacy of corticosteroid use for COVID-19 have been published. Most recently, Ma et al.'s review of 7 eligible RCTs found that corticosteroids were associated with decreased all-cause mortality (27.3 vs. 31.1%)[13]. However, in this study, dexamethasone was the corticosteroid of choice in only two of the seven trials, both of which were included in this review. Furthermore, it was stated that the survival benefit depended heavily on the RECOVERY trial, so much so that the aforementioned survival benefit was absent if the RECOVERY trial was excluded[13]. Another meta-analysis carried out by the World Health Organization (WHO) Rapid Evidence Appraisal for COVID-19 Therapies (REACT) working group also found that administration of systemic corticosteroids, compared with usual care or placebo, was associated with lower 28-day all-cause mortality[14]. However, in this study, much like Ma et al.'s review, of the 7 RCTs included in this meta-analysis, three involved dexamethasone with two of those being the RECOVERY Trial and the CoDEX Trial. Like Ma et al.'s systematic review, this meta-analysis relied heavily on the RECOVERY Trial, with 57% of the primary meta-analysis data of 28-day all-cause mortality contributed by the RECOVERY Trial[13].

Although this review indicates the quantifiable benefit of administering dexamethasone to COVID-19 patients, in particular those with invasive mechanical ventilation, some gaps in our knowledge remain. The optimum dose cannot be extracted from this analysis as various doses were used within the three studies, ranging from 6mg to 20mg IV once daily. As various doses were received, we must also recognise how this might have affected these patients differently. A paradoxical effect of a mixed anti-inflammatory and pro-inflammatory response has been associated with a high dose of corticosteroids and this might have contributed to different patient response[15]. We must also recognise that dexamethasone was not the sole treatment provided to the patients within these studies. The correct combination of other drugs and treatment that comprise SC will also have an impact on some of the previously mentioned primary outcomes. Our database search was also limited—the exclusion of Cochrane database, despite having more trials on steroid use with COVID-19, is a limitation to our study. Our screening process was limited by the exclusion of a dual author review. It should be noted that the long-term outcomes of patients were not measured. It would be appropriate to investigate this in further studies, comparing the outcomes of patients and extrapolating if the patient outcome varied by treatment type, when long term data becomes available.

It is undeniable that both the RECOVERY Trial and the CoDEX trial have had a significant role in proving the efficacy of corticosteroid—particularly dexamethasone—treatment of COVID-19 patients. Regulatory bodies such as NICE in the UK issued guidelines incorporating dexamethasone into treatment regimens for critically ill COVID-19 patients[16]. This guidance was based on the WHO's REACT working group meta-analysis. Therefore, both trials have come under scrutiny. Matthay and Thompson's critique of the "landmark" RECOVERY Trial noted that there was a lack of information provided on why 1707 patients were unsuitable for randomisation[17]. As a result, the "benefit–risk profile of corticosteroids across the full spectrum of patients with critical COVID-19 and a range of comorbidities remains uncertain"[17]. Johnson and Vinetz highlighted more evidence gaps in the RECOVERY Trial, making the point that adults requiring ventilation had a mean age of 59 years and in a post hoc subset analysis, dexamethasone did not benefit the two older age groups[18]. The efficacy of dexamethasone for older adults is therefore unclear. Less analysis has been done regarding the CoDEX trial in comparison to the RECOVERY Trial. Despite this, authors such as Salim Rezaie have mentioned the fact that the CoDEX trial was stopped early following the results of the RECOVERY trial and was underpowered as a result[19].

Conclusion

Following a systematic review of the evidence, it has been found that dexamethasone was superior to SC in patients admitted to the ICU with SARS-CoV-2 infection. Benefits included a significantly lower incidence of death in patients on invasive mechanical ventilation, higher ventilator-free days during the first 28 days and a lower overall mortality at 28 days among all patient groups. However, administration of dexamethasone to patients not on respiratory support resulted in a higher incidence of death compared to SC. ◄

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Declarations

The authors declare no conflicts of interest.

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SYSTEMATIC REVIEW

Screening and Treatment of Latent Tuberculosis: A Systematic Review of Current Evidence

Stefan Elekes[a]*, Morgan Lowe[a], Ailbhe Kenny[a], Sean Clarke[a], Charlie Eddershaw[a], Michael O'Driscoll[a], Lorraine Thong[b]

[a] *School of Medicine, Trinity College Dublin, University of Dublin, Ireland*
[b] *Trinity Translational Medicine Institute, Trinity College Dublin, University of Dublin, Ireland*
* *Corresponding author: ElekesS@tcd.ie*

Abstract

Introduction: Latent tuberculosis is an infection by the bacteria *Mycobacterium tuberculosis* where the individual affected does not have active infection or symptoms of tuberculosis infection. Individuals with latent tuberculosis infection (LTBI) remain asymptomatic and non-infectious until the bacteria become reactivated. The purpose of screening and treating LTBI is to prevent reactivation and active disease. The aim of this review is to examine the current screening criteria for LTBI, their validity, and specificity for diagnosis by looking at the currently accepted treatment options and the evidence that supports their efficacy.

Methods: Articles for review were sourced from the academic databases EMBASE and PubMed. Results were screened using PICOS criteria looking at a population of latent TB infected patients screened using a variety of screening tools.

Results: Initial database searches identified 476 articles. 19 articles fit the eligibility criteria and were included for analysis. Current screening procedures include the tuberculin skin test (TST), T-SPOT.TB, and QuantiFERON-TB (QFT-GIT) tests. Evidence showed that the T-SPOT.TB was the most cost-effective test to perform although its accuracy is not as reliable as the IGRA. Treatment plans for those with LTBI are diverse and can be beneficial in a variety of settings. The most effective treatments include isoniazid for 6 or 9 months, rifampicin for 3 to 4 months and isoniazid and rifampicin for 3 to 4 months.

Conclusion: Overall, IGRAs are the most reliable screening tests but are advised to be used in conjunction with TSTs as the TST alone has been determined to be less accurate. There are different treatment regimens, all of similar efficacy. Longer regimes were as effective than those of a shorter duration, but shorter regimes showed higher completion rates.

Keywords: Latent tuberculosis, Screening, Treatment, TST, IGRA

Introduction

An estimated one-third of the world's population are infected with *Mycobacterium tuberculosis* (MTB). Infection may be cleared by the host immune system or suppressed into an inactive form called latent tuberculosis infection (LTBI), caused by a dormant form of the bacteria that can reactivate later under favourable conditions. An estimated 2 billion people worldwide have LTBI [1].

People with LTBI are not infectious but they usually have a positive tuberculin skin test (TST) or interferon gamma release assays (IGRA) as a marker of exposure. However, neither TST nor IGRA can distinguish active TB disease from LTBI. Chest X-ray findings are usually normal or may reveal evidence of healed infection, such as granulomas or calcification in the lung and hilar lymph nodes. Such patients are at a 5–10% lifetime risk for progressing to active tuberculosis disease if LTBI is untreated[1].

The rationale for the screening and treatment of LTBI is to kill any residual dormant bacilli, thus reducing the reactivation and development of TB disease. Current therapeutic options can reduce the risk of active TB by as much as 90% if adhered to[2]. However, completion of therapy is less than 50% in many regimens due to the long duration of therapy and the risk of adverse events such as hepatotoxicity discourages patients[2]. In this paper we discuss the evidence comparing the preferred regimens for the treatment of LTBI: four months of daily rifampicin, isoniazid and rifapentine once a week for 12 weeks, three to four months of rifampicin and isoniazid, or six to nine months of daily isoniazid monotherapy.

Methods

Eligibility Criteria

We included studies meeting the following PICOS criteria:

Population: Studies that reported on patients of any age with LTBI. This included adults, children, human immunodeficiency virus (HIV) infected persons and non-HIV infected persons.

Interventions: Studies that reported on the

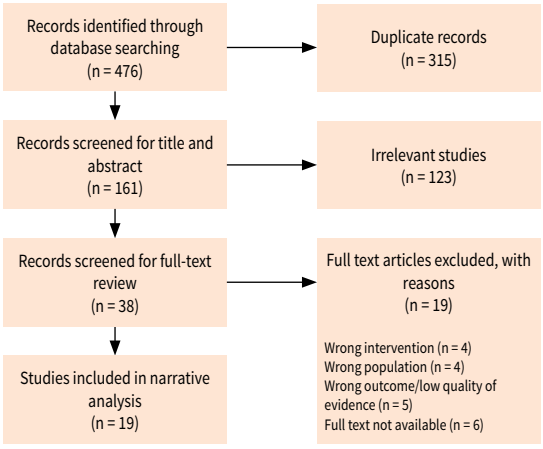
Table 1. **Search Methodology**

Search	Search Query	Filters	No. of Results
#1	(latent tuberculosis[Title/Abstract]) AND (treatment[Title/Abstract])	Meta-analysis, Randomised Control Trial, Systematic Review, English, from 2005–3000/12/12	369
#2	(latent tuberculosis[Title/Abstract]) AND (screening[Title/Abstract])	Meta-analysis, Randomised Control Trial, Systematic Review, English, from 2005–3000/12/12	107

Table 2. **Oxford Levels of Evidence (Adapted from Oxford Centre for Evidence-based Medicine Levels of Evidence, March 2009[4])**

Level	Therapy/Prevention, Aetiology/Harm
1a	Systematic Review (with homogeneity) of RCTs
1b	Individual RCT (with narrow Confidence Interval)
1c	All or none
2a	Systematic Review (with homogeneity) of cohort studies
2b	Individual cohort study (including low quality RCT; e.g. <80% follow-up)
2c	"Outcomes" Research; Ecological studies
3a	Systematic Review (with homogeneity) of case-control studies
3b	Individual Case-Control Study
4	Case-series (and poor-quality cohort and case-control studies)
5	Expert opinion without explicit critical appraisal, or based on physiology, bench research or "first principles"

Figure 1. **PRISMA Flow Diagram for Record Selection Process**



pharmacological treatment of LTBI or screening of LTBI.

Outcomes: The primary outcomes of interest were progression to active TB infection or completion of treatment. Secondary outcomes of interest included hepatotoxicity and adverse events requiring discontinuation of treatment.

Studies: The search was limited to systematic reviews, randomised controlled trials (RCTs) and meta-analyses.

Information Sources

Searches were conducted from EMBASE and PubMed. The search methodology is shown in the table in **Table 1**. The search was limited to research articles published in English and available in full text. Other exclusion criteria included obstetric populations because of differences in disease management, doses, and follow-up.

Study Selection

Duplicates were removed by comparing database search results and eliminating redundant records. The remaining records' relevance were evaluated through examination of titles and abstracts followed by the application of our eligibility criteria to full text records. Quality assessment of selected articles was done using the National Institutes of Health (NIH) screening tools for controlled interventional studies, observational cohort studies, and case-control studies[3]. Levels of evidence were assessed using the Oxford Centre for Evidence-based Medicine Levels of Evidence (**Table 2**).

Results

Database Search

The database search identified 476 citations for consideration against the eligibility criteria.

A full text review of 38 articles was performed, after which a further 19 citations were excluded. Nineteen studies were included for final analysis in which 10 detailed about screening tests for LTBI and 9 described the treatment options. The database search and exclusions are shown schematically in the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) flow diagram in **Figure 1**. Summaries of the studies can be referred to in **Table 3** and **Table 4**.

Discussion

Screening of Latent TB

TB is the number one cause of death worldwide by a single infectious agent[1], so there is a global health need for screening to be sensitive, specific, and reliable. Available screening tests include the TST and IGRA (i.e. QuantiFERON/ QFT-GIT, T-SPOT.TB).

Guidelines written by the World Health Organisation (WHO) in 2015 outline the groups of people that should be tested for latent TB. These include contacts of active TB cases, people from high-risk regions, HIV patients, patients before starting anti-tumour necrosis factor (TNF) treatment, dialysis patients, and patients prior to organ transplant[14]. There is conflicting evidence surrounding which test more effectively predicts progression to active TB. There is inadequate evidence that either test should be used over the other to predict progression from latent to active TB. Ultimately, use of either test must be influenced by availability and cost effectiveness of diagnostic tools[19].

Table 3. **Details of Records on Screening of Latent Tuberculosis**

Paper Title	Author(s), Year	Level of Evidence	Details of Study	Patient Population	Outcomes (Prognosis)	Adverse Effects
Tuberculin Skin Test - Outdated or Still Useful for Latent TB Infection Screening?[5]	Gualano et al., 2019	1A	Systematic Review (n=13)	All ages	Neither IGRAs nor TSTs can differentiate active from latent TB. TSTs will continue to be of clinical use until more accurate tests become available	N/A
The Impact of BCG Vaccination on Tuberculin Skin Test Responses in Children is Age Dependent: Evidence to be Considered when Screening Children for Tuberculosis Infection[6]	Seddon et al., 2016	2B	Individual cohort study (n=422)	Children <15	BCG vaccination had insignificant effect on TST size in children older than 5	N/A
Primary Care Screening and Treatment for Latent Tuberculosis Infection in Adults: Evidence Report and Systematic Review for the US Preventive Services Task Force[7]	Kahwati et al., 2016	1A	Report and systematic review (n=72)	18 and over	TSTs and IGRAs are moderately sensitive and very specific in countries with low TB prevalence	N/A
Management of Latent *Mycobacterium tuberculosis* Infection: WHO Guidelines for Low Tuberculosis Burden Countries[8]	Getahun et al., 2015	1A	WHO report and systematic review (n=71)	N/A	N/A	N/A
New Tests for the Diagnosis of Latent Tuberculosis Infection: Areas of Uncertainty and Recommendations for Research[9]	Menzies et al., 2007	1A	Systematic Review	All ages	Further longitudinal studies are required to define the predictive values of IGRAs	N/A
Gamma Interferon Release Assays for Detection of *Mycobacterium tuberculosis* Infection[10]	Pai et al., 2014	1A	Systematic review (n=137)	All ages	IGRAs improvement over TST is incremental rather than transformative	N/A
Screening for Latent Tuberculosis Infection: Performance of Tuberculin Skin Test and Interferon-γ Release Assays Under Real-Life Conditions[11]	Kleinert et al., 2012	1B	RCT with 1529 patients tested across 62 centres. Every patient was tested with a TST and one form of IGRA (either T. SPOT.TB or QFT)	All ages	In populations with high TB rates, the use of IGRA and TST can improve sensitivity in detecting LTBI but can also reduce specificity	N/A
Prospective Comparison of QFT-GIT and T-SPOT.TB Assays for Diagnosis of Active Tuberculosis[12]	Du et al., 2008	2B	746 suspected pulmonary TB patients across 4 hospitals in China were enrolled and diagnostic performance of QFT vs T. SPOT.TB tests were compared	All ages	The two IGRAs have similar sensitivities to aid in the diagnosis of active tuberculosis. The two assays may also be of value in diagnosis of probable TB when used in tandem High false positive rates of these diagnostic tests may limit their value in routine clinical practice in countries where prevalence of LTBI is high	N/A
QuantiFERON-TB Gold Test and T. SPOT.TB Test for Detecting Latent Tuberculosis Infection in Patients with Rheumatic Disease Prior to Anti-TNF therapy[13]	Sargin et al., 2018	2B	Individual Cohort study (n=109)	18-70 years	IGRAs are useful for detecting LTBI in patients treated with corticosteroids due to lack of correlation between corticosteroid therapy and test negativity	N/A

Table 4. **Details of Records on Treatment of Latent Tuberculosis**

Paper Title	Author(s), Year	Level of Evidence	Details of Study	Patient Population	Outcomes (Prognosis)	Adverse Effects
Diagnosing Latent Tuberculosis Infection: The 100-year Upgrade[1]	Barnes et al., 2001	2C	Report	N/A	N/A	N/A
Managing Latent Tuberculosis Infection and Tuberculosis in Children[2]	Carvalho et al., 2018	1A	Review of studies pertaining to the management of paediatric LTBI	Age 2-14	Isoniazid 9 month is preferred although rifampicin regimens are also effective. Higher doses of rifampicin may be required due to pharmacokinetics	N/A
A Systematic Review of Adverse Events of Rifapentine and Isoniazid Compared to Other Treatments for Latent Tuberculosis Infection[14]	Pease et al., 2018	1A	Systematic review (n=78)	All ages	Isoniazid and rifapentine once weekly for 12 weeks had low frequency of adverse effects. However, reporting of events was limited	Adverse effects were inconsistently reported
Isoniazid-Rifapentine for Latent Tuberculosis Infection: A Systematic Review and Meta-analysis[15]	Njie et al., 2018	1A	Meta-analysis (n=15)	All ages	3 months isoniazid and rifapentine (once weekly) had high treatment completion rates and efficacy	Similar safety profile to other LTBI treatments
Treatment of Latent Tuberculosis Infection: A Network Meta-Analysis[16]	Stagg et al., 2014	1A	Network meta-analysis	All ages	Studies were from Europe, Canada and the USA comparing 15 regimens. Effective regimens: isoniazid monotherapy 6-12+ months and rifampicin 3-4 months monotherapy or combination therapy	Regimens containing rifampicin had a lower hepatotoxicity than isoniazid only regimens. Serious adverse effects were rare in all studies. Adverse effects were infrequently recorded
Guidelines for the Treatment of Latent Tuberculosis Infection: Recommendations from the National Tuberculosis Controllers Association and CDC, 2020[17]	Sterling and Njie et al., 2020	1A	Systematic literature review with additional network meta-analysis (n=63)	All ages	Short course 3-4-month rifampicin-based treatments regimens are preferred over 6-9-month isoniazid monotherapy	Isoniazid 6-9-month monotherapies have higher toxicity risk and lower completion rates
Treatment of Latent Tuberculosis Infection: An Update[18]	Lobue and Menzies et al., 2010	1A	Systematic Review	All ages	9 months isoniazid LTBI treatment is expensive and an undesirable risk profile along with poor adherence. Rifampicin based regimens are acceptable alternatives	4 months rifampicin monotherapy has significantly lower toxicity when compared to isoniazid monotherapy
WHO Global Progress Report on Tuberculosis Elimination[19]	Harding et al., 2019	2C	WHO report	All ages	N/A	N/A
Mycobacterium tuberculosis Pathogenesis and the Dynamics of the Granuloma Battleground[20]	Rao et al., 2019	1A	Review of LTBI pathogenesis	N/A	N/A	N/A
Efficacy and Completion Rates of Rifapentine and Isoniazid (3HP) Compared to Other Treatment Regimens for Latent Tuberculosis Infection: A Systematic Review with Network Meta-analyses[21]	Pease et al., 2017	1A	Systematic review and network meta-analysis of 30 RCTs (n=88,277)	Any age confirmed with LTBI with TST and or IGRA	Shorter treatment regimens were associated with higher completion rates	N/A

Table 5. **Comparison Between TST and IGRA (QuantiFERON and T-SPOT.TB) as Screening Tools for Latent TB.**

Investigation	Specificity	Sensitivity	Cost	Follow up Required?
TST	60.3% [12]	47.8-79% [12, 13, 14]	Low [6]	Yes [9]
QFT-GIT	85.7-97.7% [12, 13]	73.6-96% [12, 13, 14]	High [6]	No [6]
T-SPOT.TB	73.5-92.5% [12, 13]	66.7-96% [12, 13, 14]	High [6]	No [6]

TSTs are useful for initial investigations because they can require less equipment and are cheaper but they can also be unreliable[21,22]. TSTs are considered to be inaccurate in individuals with other conditions, especially autoimmune diseases, as the skin test is an interpretation of the body's reaction to the injection of the tuberculin in the skin. This can result in false positives in the skin test, due to an error in interpretation or a reaction to the injection rather than the tuberculin itself[5]. Another downfall of TST is that they require multiple clinic visits for interpretation. The inaccuracy of TST and the difficulties associated with multiple visits to the clinic for monitoring were identified by several studies[15] **(Table 5)**.

IGRAs are widely known to be more accurate markers than TST and present advantages such as high specificity, high sensitivity, and easier administration and monitoring, as an *ex vivo* blood test. T-SPOT.TB and QFT-GIT are both techniques for detecting interferon-gamma (IFN-γ). T-SPOT.TB is a measure of the amount of IFN-γ released from peripheral blood mononuclear cells and QFT-GIT measures the concentration of IFN-γ in whole blood. As IFN-γ can be released by T cells in response to many infections and not just against MTB exposure, the possibility of false positives cannot be ruled out[15,16]. Disadvantages include the high cost compared to TST, the need for specific laboratory equipment, and the lack of specific criteria for analysing results **(Table 5)**. When comparing the performance of TST and IGRAs, it is important to know the Bacillus Calmette–Guérin (BCG) status of the patient as this can influence the outcome of the TST result[17]. While IGRA tests are more reliable, the most effective method to get the most accurate result is to perform both TSTs and IGRAs[21]. However, IGRAs have decreased specificity in countries with a high incidence of latent tuberculosis, so it cannot be used as a confirmatory diagnostic tool but rather as a negative prognostic marker[11]. IGRAs can rule out TB but may not be able to confirm it as different types of testing and investigations are needed to do so.

Treatment of Latent TB

Treatment of LTBI is indicated for those at increased risk for progression measured by risk factors as seen in the patient's history and previous IGRAs or TSTs[11]. Patients with LTBI are considered effectively treated if they were administered ≥1 medication to which their TB strain was likely susceptible[2]. Currently, no gold standard of treatment is recognised, however, there are five treatment regimens that have been proven to be effective when compared to no treatment/placebo[8]. These regiments are:

- Isoniazid (INH) daily for 9 months
- INH daily for 6 months
- INH + rifampicin (RIF) for 3-4 months
- RIF daily for 4 months
- INH with rifapentine once weekly for 12 weeks

Shorter regimens are also known to have similar efficacy, favourable tolerability, and higher treatment completion rates[19]. Some patients with HIV cannot undergo the shorter regimens due to interactions with antiviral drugs, and therefore two alternative monotherapy options are included in the discussion.

Based on analysis of the current academic evidence, the dose of the drug is not as significant to the results as the timeframe of the drug regimen. INH daily for 6 months and daily for 9 months were often not completed. INH daily for nine months is the regimen recommended for all groups. In many studies INH daily for 9 months was used as the standard comparator[19]. INH reduces the risk of active TB by as much as 90% if taken daily for nine months[2]. Hepatotoxicity of INH is up to 2.9%[3], therefore, long duration of treatment is not always advantageous. Close monitoring and surveillance are strongly suggested[2]. INH daily for 9 months showed a statistically significant benefit in preventing active TB compared to a placebo[12]. This treatment is recommended in patients aged 2-11[8]. INH daily for 6 months had similar efficacy to INH daily for 9 months but greater adherence due to a shorter course of treatment[12].

Three to four months of INH with RIF had decreased frequency of hepatotoxicity, though discontinuation due to adverse effects was more common[19]. The adverse event profile of INH with RIF for three months had an increased frequency of flu-like reaction, but lower frequency of hepatotoxicity[7].

RIF monotherapy for four months duration had an equal effect and lower rates of hepatotoxicity when compared to other treatments of six or more months[5]. The regimen is strongly recommended for HIV negative adults, and children of all ages. The four-month monotherapy of RIF had better compliance when compared with INH daily for 9 months[19].

Combination INH with rifapentine once weekly for 12 weeks allowed a shorter time frame of treatment and can be used in individuals with HIV who are not prescribed antiretrovirals[8]. Rifapentine with INH had equivalent effectiveness and lower hepatotoxicity than INH daily for 6 months[5].

Regimens containing RIF are considered more effective than INH monotherapy when considering adverse effects and cost[5]. Treatment completion rates

were higher with the three-month regimen at 87.5%, compared with other regimens at 65.9%.

RIF-Pyrazinamide combination is not recommended due to adverse effects, including hepatotoxicity in HIV-negative individuals. However, this combination appears to be safe in HIV positive individuals.

Poor adherence was found to be the most common reason for the failure of LTBI treatment. The choice of treatment regimen for LTBI will depend on the clinician's assessment of the likely adherence level of the patient, antibiotic susceptibility of the presumed source case, drug tolerance, and overall feasibility.

There were several limitations to this research. One limitation found during the research phase was that most papers came out of the United States of America rather than Ireland or other European countries, so the data found may be less relevant to Ireland as well as the rest of the world. Also, it is important to note is that comparison of regimen adverse events was limited to hepatotoxicity as this was the only toxicity consistently compared across studies, and this review does not account for variation in duration of follow up across studies. Studies with longer follow up are expected to have a higher incidence of adverse events.

Conclusion

Tuberculosis remains as one of the most important infectious diseases with a high mortality rate. We reviewed up to date studies on the effectiveness of various screening and treatment methods and identified some successful tests and agents that can yield better detection of LTBI and better clinical outcomes. Overall, IGRAs are the most reliable screening tests but are advised to be used in conjunction with TSTs as the TST alone has been determined to be less accurate. High risk groups that should be screened for LTBI are those infected with HIV, infants, children, those prescribed with immunosuppressants, and the elderly. Treatment plans for those with LTBI are diverse and can be beneficial in a variety of settings. The most effective treatments include isoniazid for 6 or 9 months, rifampicin for 3 to 4 months, isoniazid and rifampicin for 3 to 4 months. Shorter treatment regimens are more effective than longer regimens due to higher treatment completion rates. These findings are important as they represent valuable diagnostic and therapeutic strategies that could be used to guide policies and improve the prognosis of those with LTBI.

Contributorship Statement

Morgan Lowe, Ailbhe Kenny, Sean Clarke, Charlie Eddershaw, and Michael O'Driscoll all contributed equally to this work.

Declarations

The authors declare no conflicts of interest. Stefan Elekes holds the position of feature writer on the editorial committee of the TSMJ Volume 21. This article was anonymised following submission and subsequently reviewed and accepted by an independent team of editors and peer reviewers as per the TSMJ's peer review and article acceptance protocol.

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SYSTEMATIC REVIEW

Management of Hydrocephalus in Congenital Toxoplasmosis using Pyrimethamine and Sulfonamide: A Systematic Review

Sarah Waicus[a]*, Lauren Vrbanic[a]

[a] *School of Medicine, Trinity College Dublin, University of Dublin, Ireland*
**Corresponding author: WaicusS@tcd.ie*

Abstract

Background: Congenital toxoplasmosis is a serious disease that occurs when the foetus is infected with the parasite *Toxoplasma gondii*. A consequence of vertical transmission from mother to foetus is hydrocephalus. This is classified as an increase in intracranial pressure causing swelling of the brain. It is unknown whether the current gold standard of antibiotic treatment of pyrimethamine and sulfonamide is adequate. The objective of this review is to compare the efficacy of pyrimethamine and sulfonamide treatment duration in managing hydrocephalus induced by congenital toxoplasmosis.

Methods: A systematic review was conducted by two independent reviewers across several medical databases including Ovid MEDLINE, Cochrane Central and EMBASE. Seven articles including case reports, retrospective cohorts, randomised controlled trials, longitudinal studies, and systematic reviews met the inclusion criteria. Infants were classified from birth to 24 weeks old.

Results: There was a lack of conclusive evidence regarding the efficacy and safety of pyrimethamine and sulfonamide. Multiple studies revealed pyrimethamine and sulfonamide were effective in reducing infant deformities and neurological conditions, only when rapidly administered after birth. However, contradicting evidence revealed pyrimethamine and sulfonamide had no significant effect on hydrocephalus.

Conclusion: Novel pharmaceutical interventions for managing hydrocephalus caused by congenital toxoplasmosis are needed, as the existing treatments are inadequate. Since treatment options have dwindled in the last decade, toxoplasmosis is classified as a neglected parasitic infection. Renewed interest in conducting higher-quality trials is required to elucidate different therapeutic interventions for clinical use.

Keywords: Disease transmission, Hydrocephalus, Antibiotics, Congenital toxoplasmosis

Background

Toxoplasmosis is a zoonotic infection caused by the *Toxoplasma gondii* parasite[1]. Replication of the parasite occurs in the domestic cat. The cat sheds *Toxoplasma gondii* oocytes in its faeces into the environment. The oocytes can then be ingested by other warm-blooded animals or transmitted to humans through contaminated food, particularly undercooked meat, and soil. Toxoplasmosis is of critical public health importance, as it affects one-third of the global population with a seroprevalence in industrialised countries estimated to be between 10%–50% and around 80% in tropical areas with poor sanitation[2]. Toxoplasmosis can present with many non-specific symptoms, making it difficult to diagnose.

Toxoplasmosis can be vertically transmitted across the placental barrier, which can have severe health consequences for the developing foetus[1]. Early diagnosis of congenital toxoplasmosis through polymerase chain reaction (PCR), anti-*Toxoplasma* antibodies using enzyme-linked immunosorbent assay (ELISA), and IgG and IgM *Toxoplasma*-specific antibody detection can greatly improve the health of both mother and foetus, as interventions can be started immediately[3]. Symptoms of severe congenital toxoplasmosis in the neonate include blindness, intellectual disability, intracranial calcifications, and hydrocephalus. Hydrocephalus is an abnormal increase in intracranial pressure and expansion of the ventricles. This causes an increase in head circumference, irritability, vomiting, and sutural diastasis in the newborn[4].

Ventriculomegaly, a condition that can lead to hydrocephalus, can be observed on a prenatal ultrasound at 18–20 weeks' gestation[4]. Termination of pregnancy is recommended to mothers with a diagnosis of congenital toxoplasmosis before 26 weeks of gestation in France[5]. Antibiotic treatments for suspected cases of congenital toxoplasmosis include administration of pyrimethamine and sulfonamide (P/S), both of which inhibit parasitic folate metabolism[6]. Sulfonamide acts as an inhibitor to the dihydropteroate synthetase enzyme, whereas pyrimethamine inhibits the dihydrofolate reductase enzyme. The objective of this review is to compare the efficacy of P/S antibiotic duration of treatment in managing hydrocephalus induced by congenital toxoplasmosis. These results have the potential to aid in expanding the national guidelines in obstetrics and gynaecology for the Royal College of Physicians of Ireland.

Methods

A systematic review was conducted by two independent reviewers utilising Ovid MEDLINE, Cochrane Central, and EMBASE. The keywords "congenital toxoplasmosis," "pyrimethamine," "sulfadiazine," and "hydrocephalus" were used in the search. Eligibility assessment was performed by the independent reviewers and disagreements were resolved by consensus. A data extraction Excel sheet was developed and used to compile and summarise the relevant studies. The inclusion criteria were established in line with the study objective where relevant articles underwent data extraction and analysis. Full electronic search history can be found in the Preferred Reporting Items for Systematic Reviews and Meta Analyses (PRISMA) chart in **Figure 1**.

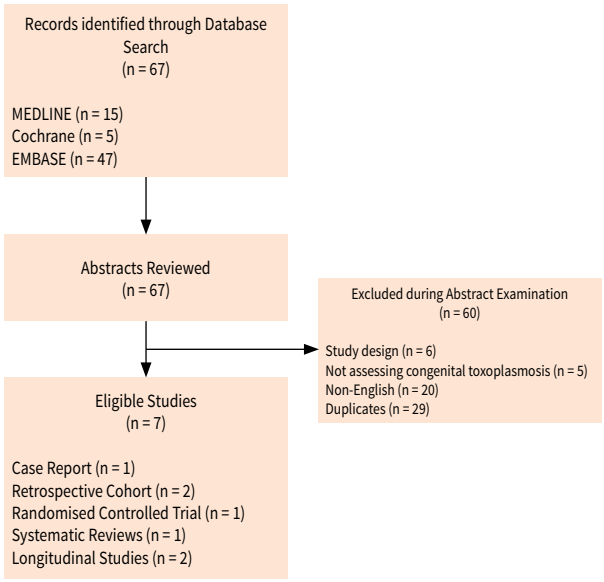
Inclusion and Exclusion Criteria

Qualitative, quantitative, cohort, and case studies written in English from January 1990 to November 2020 describing the use of antibiotic treatments for infants with hydrocephalus caused by congenital toxoplasmosis were included. The infant period considered was from birth to 24 months old. Mothers that were treated prenatally with P/S were also included in the review. The Population, Intervention, Comparison, and Outcome (PICO) tool was used to inform and guide the keywords and inclusion criteria used in the search. Studies published before January 1990 and after November 2020 were excluded. Foreign-language articles, and articles not assessing hydrocephalus or congenital toxoplasmosis as the primary focus were excluded.

Search Results

Relevant articles underwent data extraction and analysis. A search yielded 67 results (15 in Ovid Medline, 47 in Embase, 5 in Cochrane) that matched the predefined search parameters. Of the 67 articles identified in the search strategy, 7 articles met the final inclusion criteria and warranted analysis. 60 articles were excluded: 6 were commentary articles not adhering to the study design, 5 were not directly assessing congenital toxoplasmosis, 20 were non-English, and 29 were duplicates between the database searches. 7 articles met the inclusion criteria, including: 1 case report, 2 retrospective cohorts, 1 randomised controlled trial, 2 longitudinal studies, and 1 systematic review were included. Corroborative themes were identified and reported in the outcomes table in **Table 1**.

Figure 1. **Chart of Search History of Congenital Toxoplasmosis in Neonates with Hydrocephalus**



Results

The seven articles that were included in the final criteria revealed conflicting results on the efficacy of P/S in treating congenital toxoplasmosis. Hydrocephalus was examined as the main outcome.

Support of P/S Antibiotic Use

A longitudinal study conducted by McAuley et al. examined 44 children with congenital toxoplasmosis over ten years[12]. According to this study, developmental, neurological, and ophthalmological deformities including hydrocephalus were decreased with rapid administration of P/S, with a relatively low frequency of neutropenia being the only haematological toxicity observed. Dosage of pyrimethamine and sulfadiazine varied, given in milligrams for half the infants weight in kilograms[12]. After P/S was discontinued, 3 children developed retinal lesions and afebrile seizures, however, authors concluded that rapid P/S therapy was successful in treating neurological deformities, including hydrocephalus[12].

Subsequently, Roizen et al. conducted a one-year longitudinal study that examined 36 infants from 1–13 months of age with congenital toxoplasmosis[11]. In this study, infants were treated with P/S for one month, one year, or not treated at all where dosage varied considerably between each infant depending on weight. The authors in this study found that children with hydrocephalus ex vacuo performed poorly on neurological and developmental tests. Neurologic and developmental outcomes were significantly reduced in children treated with P/S in comparison to those untreated or temporarily treated for less than a month ($p=0.001$) and children treated with P/S for the full year had improved neurological scores[11].

Foulon et al. expanded on the early neurological studies and examined the efficacy of P/S against the antibiotic spiramycin given antenatally to mothers[10]. In this study, prenatal doses of P/S ranged from 25–500 mg against 1 g of spiramycin. Multivariate analysis revealed that rapid prenatal treatment led to a significant reduction ($p=0.021$) in the severity of physical defects, including hydrocephalus and administration of P/S or spiramycin was predictive of the absence of sequelae ($p=0.026$)[10]. However, there was no difference in the efficacy between P/S compared to spiramycin[10].

Opposition of P/S Antibiotic Use

A large retrospective cohort study conducted by Gras

Table 1. **Outcomes of Pharmacological Interventions of Congenital Toxoplasmosis**

Study	Type	Population	Drug Used	Conclusion
Mandelbrot et al., 2018[7]	RCT	143 infants with congenital toxoplasmosis	Combination of P/S	Lower transmission of toxoplasmosis to the foetus when using P/S but did not reach statistical significance. No foetal cerebral toxoplasmosis lesions in the group.
Tamaru et al., 2011[8]	Case study	1 infant with severe congenital toxoplasmosis	Combination of P/S	An infant with severe hydrocephalus was treated seven days after birth with P/S. Side effects were observed, and treatment was terminated. New foetal drug therapies for treatment of congenital toxoplasmosis are suggested to reduce maternal and foetal risks.
Gras et al., 2001[9]	Retrospective cohort	181 liveborn infants with congenital toxoplasmosis	Combination of P/S against spiramycin or no treatment	No significant effects were found when using P/S on intracranial, pericranial, or ocular lesions by age 3.
Foulon et al., 1999[10]	Retrospective cohort	64 infants with congenital toxoplasmosis	Combination of P/S	Early treatment commencement resulted in a significant reduction (p=0.021) in the number of severely affected infants with hydrocephalus. P/S in combination did not have different effects from spiramycin.
Roizen et al., 1995[11]	Longitudinal study	36 infants followed to ten years of age with congenital toxoplasmosis	Combination of P/S	Neurologic and developmental outcomes were significantly reduced in children treated with P/S in comparison to those untreated or temporarily treated for less than a month (p=0.001).
McAuley et al., 1994[12]	Longitudinal study	44 infants followed to 1 year of age with congenital toxoplasmosis	Combination of P/S	P/S was concluded as a feasible treatment for infants under the age of 1. The toxicity of administered P/S was minimal and manageable. The relatively low frequency of neutropenia was the only significant form of haematologic toxicity observed.
Peyron et al., 1999[13]	Systematic review	594 children variable ages with congenital toxoplasmosis	Combination of P/S	Treatment of pregnant women with P/S showed inconclusive results in the vertical transmission rate of congenital toxoplasmosis.

et al. revealed no difference in the efficacy between P/S, spiramycin or no treatment when P/S was given antenatally in 181 new-borns[9]. In this study, mothers were prescribed 50 mg/day of pyrimethamine and 3 g/day of sulfadiazine after confirmation of infection with seroconversion of IgG and IgM antibodies and after birth neonates were immediately prescribed with pyrimethamine (3 mg/kg/3 days) and sulfadiazine (75 mg/kg/day) for three weeks. Results revealed no effect of P/S on intracranial, pericranial, or ocular lesions by three years[9].

A case study of a seven-day old neonate with congenital toxoplasmosis was administered a combination of P/S postnatally in addition to antenatal treatment[8]. In this study, during the mother's pregnancy, she was given oral administration of azithromycin in addition to P/S and acetylspiramycin starting at 23 weeks gestation. Postnatally, Tamaru et al. observed serious side effects including hepatosplenomegaly, intracranial calcifications, meningitis, and ascites[8]. The authors of this study hypothesised that these side effects could have been caused by the teratogenic effects of pyrimethamine during the first trimester of pregnancy.

A systematic review conducted by Peyron et al. examined different treatments for congenital toxoplasmosis including P/S, spiramycin, azithromycin, or no treatment[13]. The authors of this study concluded that there was a lack of sufficient evidence that antenatal P/S had any positive effects on the foetus. Recent research has shed further conflicting evidence on the use of P/S. A novel randomised controlled trial (RCT) conducted by Mandelbrot et al. examined the antenatal treatment for mothers with P/S in comparison to spiramycin[7]. In this study, there was a lower transmission rate observed with P/S treatment, however, these results did not reach statistical significance and there were no foetal cerebral toxoplasmosis lesions when treated with P/S.

From the results obtained, there was a lack of conclusive evidence regarding the efficacy and safety of P/S treatment for infants diagnosed with hydrocephalus caused by congenital toxoplasmosis. Multiple studies using P/S were shown to be effective in reducing infant deformities and neurological conditions when rapidly administered after birth[10–12]. However, contradicting results elucidated that combination therapy did not affect intracranial lesions or deformities caused by congenital toxoplasmosis[9]. This could be due to the reduced efficacy of the drug itself or the timing of the treatment. The lack of conclusive evidence against using P/S as a treatment for hydrocephalus can be attributed to the different methods used in various studies. Many of the studies used direct imaging techniques to visualise the progression of hydrocephalus such as ultrasonography, radiography, computed tomography, and magnetic resonance imaging[8–10]. However, in the studies conducted by Roizen et al.[11] and McAuley et al.[12] used indirect methods such as neurological and blood serum tests were used to measure the effects of hydrocephalus. The Health Service Executive (HSE) Ireland's guidelines to diagnose hydrocephalus requires an occipitofrontal circumference measurement greater than 38 cm followed by an immediate cranial ultrasound[14]. The inconsistent measurement of hydrocephalus between these studies contributes to the inability to draw meaningful and concrete conclusions from the data.

Reducing the transmission rates of congenital toxoplasmosis with P/S was also investigated. Data collected by Peyron et al.[13] recognised that there was inconclusive evidence on whether the rate of transmission of congenital toxoplasmosis was lower when pregnant

women were treated with P/S[13]. Furthermore, the only high quality RCT conducted by Mandelbrot et al.[7] showed a reduction in the vertical transmission from mother to foetus, however, the results were not statistically significant[7]. Due to conflicting results, it remains unknown whether treatment with P/S during pregnancy reduces the transmission of congenital toxoplasmosis.

The safety of P/S treatment was examined and revealed conflicting results. The P/S treatment on both mother and infant was concluded to be safe in managing the infection and had few minor adverse effects[12]. Neutropenia was a side effect, however, the authors noted that this could easily be treated with other drugs, and therefore was not considered a serious adverse reaction[12]. In contrast, a recent case study by Tamaru et al.[8] revealed adverse side effects from treatment in both mother and foetus. Side effects of this study included: bone marrow suppression, hepatotoxicity, meningitis, intracranial calcifications, and ascites. Therefore, in this study, P/S was not recommended as a treatment[8]. The results of these studies prompt further investigation into the non-neurological P/S side effects.

Discussion

There is an apparent lack of conclusive evidence regarding the safety and efficacy of P/S on the prevention and treatment of hydrocephalus caused by congenital toxoplasmosis. The results of various study types cannot be accurately compared to each other to draw results from this collection of studies. Therefore, no formal conclusions can be made from the data.

Toxoplasmosis is classified as a neglected parasitic infection in the United States[15]. Therefore, there is a limitation on the volume and quality of current research. Most investigations on *Toxoplasma gondii* were conducted in the 1990s when the disease was newly discovered. Since then, there has been a decrease in research and interest on this topic, which has led to a lack of epidemiological data. Hence, the conclusions that can be drawn from this information are based on limited amounts of research. Furthermore, this lack of research has diminished progress in the development of novel pharmaceutical therapies to treat congenital toxoplasmosis. Consequently, patients are still being treated with P/S, which has remained the gold standard for decades.

Another limitation of this review is that most articles had a small sample size, which affects the generalisability of the studies. The results are relevant for the sample, however, may not be able to be extrapolated to an entire population. The studies included in this systematic review are also limited, as they are mainly longitudinal and retrospective in their design, rather than RCTs. RCTs are the gold standard in evidence-based medicine due to the control for bias by randomisation and should be the goal in determining treatment efficacy[16]. Additionally, the P/S therapy was not compared to a single dose of each independent drug. Therefore, the efficacy of pyrimethamine and sulfadiazine on their own in comparison to the combination of P/S is unknown. A final limitation of this review is that the studies all used the same combination of drugs but did not compare the treatment efficacy to the same control group. For example, Foulon et al.[10] compared the efficacy of P/S to another pharmacological treatment of spiramycin, whereas Roizen et al.[11] compared the efficacy of the drug combination to untreated patients.

Conclusion

Further research on novel pharmaceutical interventions for hydrocephalus caused by congenital toxoplasmosis is warranted. An increased interest in conducting RCTs for various combinations of treatments and antiparasitic drugs are required to obtain objective data for clinical use. Some examples of alternative antiparasitic drugs that can be included in combination therapy are spiramycin and clindamycin, both of which are bacteriostatic agents[7,17]. As pyrimethamine has shown teratogenicity in the first trimester of pregnancy, the safety and efficacy of novel drugs should be researched during this time[18]. The development and use of antiparasitic drugs pose challenges for various parts of the world. Therefore, it is required to investigate which pharmaceutical interventions are the most appropriate options for populations of various socioeconomic and geographic backgrounds.

A critical effort must be made to increase public awareness of congenital toxoplasmosis in both endemic and non-endemic areas. This not only includes education for pregnant women, but also for women of childbearing age. An 2008 article written by Elsheika suggests multiple approaches should be taken to prevent congenital toxoplasmosis[19]. This includes informing and educating local health officials so accurate information can be passed onto pregnant women[19]. This is especially critical in endemic and resource-poor regions, as it is a low-cost intervention. Another preventative measure suggested in this paper is the implementation of screening programmes for pregnant mothers so interventions can be started immediately if they are infected with the parasite.

Countries in Africa and South America do not routinely screen for possible congenital toxoplasmosis infections, as it can be costly for these populations of lower socioeconomic status[20]. However, this is a global obstacle, as countries in Europe and North America do not currently have long-term routine national screening programmes. There have, however, been temporary screening programmes. In Ireland, a two-year pilot newborn screening programme was initiated in 2005, which involved a two-step IgM dried heel test 72–120 hours after birth[21]. The programme found early signs of visual and neurodevelopmental signs for postnatal infants. As a result, screening allowed immediate treatment that was faster. Currently, in France, there is a national screening programme called *ToxoSurv*, which can diagnose antenatal and post neonatal toxoplasmosis infection up to one year of age. ToxoSurv and previous programmes dating back to 1995 have been associated with a decrease in seroprevalence of toxoplasmosis[22].

With increased research on congenital toxoplasmosis, the ultimate goal is the development of a vaccine, which would decrease transmission of the parasite.

Currently, there are no human vaccine candidates for *Toxoplasma gondii*, as it shows a large degree of variability and antigenic polymorphism[23]. Since there is no imperative for a vaccine at this current time, it is important to elucidate successful pharmacological alternatives. Due to the lack of evidence supporting P/S as a treatment for hydrocephalus caused by congenital toxoplasmosis, future research is required to discover novel interventions. ◄

Declarations

The authors declare no conflicts of interest. Sarah Waicus holds the position of production director on the editorial board of the TSMJ Volume 21. This article was anonymised following submission and subsequently reviewed and accepted by an independent team of editors and peer reviewers as per the TSMJ's peer review and article acceptance protocol.

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LITERATURE REVIEW

Bacteriophage Therapy for Treating Infections: Hope or Hype?

Anurag Nasa

School of Medicine, Trinity College Dublin, University of Dublin, Ireland (NasaA@tcd.ie)

Abstract

Bacteriophage therapy employs the use of viruses to kill bacteria and has been noted to confer reversal of antimicrobial resistance. It was proposed around the same time as antibiotic therapy for combatting infections but lost the race for becoming the mainstay therapy. However, antibiotic resistance is increasingly resulting in morbidity and mortality. Bacteriophage therapy as an alternative approach for combatting infections has garnered speculation and interest of many scientists with hopes that it may become a management strategy for multi-drug resistant infections.

The aim of this review is to shed light on the developments in bacteriophage therapy, explain lytic cycles as the proposed functional mechanism and discuss the evidence base: preclinical, case-based and clinical trials.

There is preliminary evidence that alludes to an element of safety and efficacy in treating multidrug resistant infections. However, there is a paucity of high-quality evidence, which could bring this therapy into routine practice. This is further burdened by limitations such as the need for an individualised approach and our lack of understanding of the immune reactions to it. This therapy is quite promising, but much work is needed before it can be considered for routine clinical practice.

Keywords: Bacteriophage, Infection, Antibiotic, Antibiotic resistance, *Pseudomonas aeruginosa*

Introduction

Following discovery of Penicillin in 1945, Alexander Fleming warned us that "it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them"[1]. Now, we rely very heavily on the use of antibiotics. We use them prophylactically pre-operation and post-transplant, pre-emptively, empirically, and definitively, making modern medicine without antibiotics unimaginable. Yet we are faced with the very threat we were warned about: antimicrobial resistance[2].

Resistance to antibiotics has become a crisis, especially in certain bacterial pathogens where there is a newfound paucity of therapeutic alternatives and a marked prevalence of pan-resistant strains[3]. Antimicrobial resistance is the cause for an estimated 700,000 deaths annually; projected to escalate to 10,000,000 by 2050 without action[4,5]. It is imperative that other non-antibiotic options are explored. One potential solution is bacteriophage therapy: the killing of bacteria using viruses.

Bacteriophage therapy seems very promising for two main reasons. Firstly, it has bactericidal effects specific to the bacterium being targeted. Secondly, there have been claims that it can potentially reverse antibiotic resistance, indicating a prospective role as a complement or adjunct to therapy with antibiotic therapy[6]. As such, the aim of this review is to shed light on the developments in bacteriophage therapy, explain lytic cycles as the proposed functional mechanism and discuss the evidence base: preclinical, case-based and clinical-trials.

Discovery and Use in the Last Century

Bacteriophages are viruses whose existence was first postulated in 1896 by an English bacteriologist, Ernest Hanbury Hankin. Félix d'Herelle, a French microbiologist, finally discovered this mystery entity in 1917 when he analysed stools from patients recovering from bacillary dysentery. He had isolated what he referred to as an "invisible microbe," a filterable virus which infects Shiga bacilli and is able to lyse them—"a virus parasitic on bacteria". This was named "bacteriophage" after presentation to the Académie des Sciences in September that year[7].

This area seemed welcome initially, given that infectious diseases were decimating populations. Eventually, it was not given attention unlike its antimicrobial counterpart, antibiotics, after their discovery in 1928[2]. Antibiotics had broader spectra and their ease of use was unmatched as compared to bacteriophages. Antibiotics thrived while the bacteriophage therapy was forgotten about in all parts of the world except some. They were adapted in the

former Union of Soviet Socialist Republics with the development of the Eliava Institute in Tbilisi, Georgia. This centre for bacteriophage therapy still exists in the modern day, and it is not the only one. There are centres in other countries too such as Poland and Belgium; with Belgium being the first country to make bacteriophages available in pharmacies based on their magistral phage regulatory network[7].

Bacteriophage Biology

Classified based on genomics and morphology, the diversity between bacteriophage species is remarkable.

They are known to exhibit two primary cycle types: lytic and lysogenic.

Lytic cycles result in destruction of bacterial cells. The mechanism involves entry into a bacterium, replication, protein synthesis, assembly, and colonisation. The bacteriophage injects its DNA into a bacterium, which gets replicated using the bacterial nucleotides. The bacteriophage DNA then uses bacterial cellular resources to synthesise proteins conducive to its cloning. After they have colonised the cell, they secrete hydrolytic enzymes—called endolysins—to cleave the host bacterium's cell wall and infect other bacteria. This is the primary mechanism by which the proposed therapy with bacteriophages can kill bacteria and clear infections.

Lysogenic cycles, on the other hand, involve the integration of the viral DNA into the host bacterial DNA. When integrated into the bacterial host's DNA, the bacteriophage is referred to as a "prophage". This is a period where they are inactive and replicate harmlessly coupled with the bacterial cell. This pathway also has the capacity to convert to the lytic cycle under certain stressful circumstances. If stressful cellular environments are present, the bacteriophage DNA will excise from bacterial host DNA and initiate bacterial destruction using the lytic cycle.

Lambda phages are a well-known example of temperate bacteriophages—species that can develop using both lytic or lysogenic pathways depending on cellular environment. They infect the species *Escherichia coli*, and a lot of our knowledge regarding the molecular mechanisms of lysogeny are based on the study of it[8-10].

Combatting Antibiotic Resistance

Resolution of the rapidly rising antimicrobial resistance potentially lies in the use of bacteriophages. This was demonstrated in an article by Chan et al., published in *Scientific Reports* in 2016[6].

Some bacteria can gain resistance to antibiotics via their multi-drug efflux (Mex) systems. Interestingly, bacteriophages can infect bacteria by entering via those Mex systems. As such, Chan et al. focused on the effect of bacteriophages on this efflux pump resistance mechanism against antibiotics[13]. It was hypothesised that a bacteriophage, which binds to the outer-membrane protein of Mex (OprM), would cause the host bacteria it colonises to evolve. This evolution would involve downregulating the expression of the Mex system, and though the bacteria may gain resistance to the bacteriophage in this manner, success would still be achieved. This is because expression of efflux proteins would downregulate the bacteria, and antibiotic sensitivity would return.

To test this, Chan et al. obtained samples of naturally occurring bacteriophages from locations such as sewage, soil, lakes, and rivers[13]. In this study, they identified 42 species that were able to infect the PA01 and PA14 strains of *Pseudomonas aeruginosa* (an opportunistic gram negative, rod-shaped bacterium, increasingly seen to be pandrug-resistant[11]). In addition, a unique lytic bacteriophage from the family *Myoviridae* was identified in a freshwater lake in Connecticut, USA and given the name OMKO1. This was the only bacteriophage that infected the multidrug resistant (MDR) bacteria by binding to the OprM protein of the efflux pump.

Chan et al. then observed something wonderful after further investigation: a genetic trade off. The bacteriophage-sensitive bacteria tend to efflux antibiotics but get killed by the virus. The phage-resistant mutants have impaired drug efflux ability which makes them vulnerable to antibiotics that are typically useless against this bacterium. The paper elucidated a mechanism by which bacteriophages can increase sensitivity to antibiotics[6].

Preclinical Evidence

A review article published by Melo et al. in February 2020 discussed the efficacy of bacteriophage therapy based on 10 years of preclinical studies, including mostly the studies on murine models[12]. The infections at the focus of this study were broad and were grouped into various categories: skin and soft tissue, eye and ear, respiratory tract, gastrointestinal and urinary tract. Regarding skin and soft tissue infections, efficacy was demonstrated against common pathogens when the phage-based concoctions were applied topically and to a lesser extent using some other routes of administration in this study. Clear evidence of efficacy was documented from application of the therapy across all the other groups as well; however, it was mentioned that many studies produced varying infection clearance between subjects[12]. This study was important in demonstrating that bacteriophages have the capacity to become our asset for infection clearance in the future. However, the demonstrated variation in efficacy between subjects receiving the same therapy is undesirable[12]. Trials in human subjects are required to confirm if these results can be replicated in clinical use.

Case Report–Based Appraisal

A 76-year-old man in 2012 documented by Chan et al. had an aortic aneurysm treated by aortic arch replacement surgery and a post-surgical complication was the infection of the graft and mediastinum by *P. aeruginosa*[13]. In this case report, practical algorithms in place and applied back then were the use of systemic antibiotics, debridement of infected tissue and graft excision. The patient in this report had numerous recurrences in the subsequent years, the management of which was aided by antibiotics. This led to increased resistance and there

was also biofilm formation noted, which decreases penetrance of antibiotics. A solution containing OMKO1 bacteriophages and ceftazidime were applied into the mediastinal fistula and the patient was discharged home and four weeks post-surgery, the 76-year-old patient suffered an aortic perforation. However, the only thing which the cultures revealed was *Candida* species, and it was confirmed that recurrence of *P. aeruginosa* infection had not occurred[13].

A separate case report documented by Schooley et al. illustrated outcomes from treatment of an MDR *Acinetobacter baumanii* infection using bacteriophages[14]. This case describes management of a 68-year-old man with necrotising pancreatitis complicated by MDR *Acinetobacter baumanii* infection on a background of diabetes. The 68-year-old patient was comatose, and the infection was resistant to last line antimicrobials such as colistin. Bacteriophage therapy was initially administered to the patient as a cocktail (ΦPC). 36 hours after the initial administration of the therapy, a new cocktail (called ΦIV) was administered intravenously alongside minocycline and repeated frequently over the next 48 hours. The patient then recovered from the coma after several weeks and improvement was seen on all fronts over the next three weeks, and the patient was discharged[14].

There have also been numerous other case studies demonstrating bacteriophage efficacy. Two of these were published towards the end of 2019. Maddocks et al. demonstrated efficacy of the therapy in treating pneumonia and empyema caused by *P. aeruginosa* in a 77-year-old lady with hypersensitivity reactions elicited on administration of numerous antibiotic types[15]. In this case study, the patient had exhausted antibiotic therapy extending to meropenem, which she had developed resistance to. AB-PA01 bacteriophage therapy was initiated as an adjunct to the gentamicin and ciprofloxacin. The patient's status improved rapidly from this treatment as she had improved oxygenation and cessation of sedation and, within a week, the patient was stepped down from the intensive care unit to the high dependency unit[15].

A study by Law et al. was published in the same year and demonstrated the use of bacteriophage therapy in a 26-year-old cystic fibrosis patient who developed MDR *P. aeruginosa* pneumonia with respiratory failure[16]. This 26-year-old patient had been placed on colistin previously, which led to renal failure, AB-PA01 bacteriophage therapy was initiated as an adjunct to ciprofloxacin and piperacillin-tazobactam. The patient became afebrile by the seventh day of the therapy and ambulatory by the eighth week and monitoring of the first 100 days post therapy revealed no infective recurrence[16].

Although these findings make bacteriophage therapy appear lucrative, it is salient to note that the positive effect evidence demonstrated here might be due to exceptional circumstances. There is an array of variables that could be at play, and it cannot be assumed that bacteriophage therapy caused these effects alone.

Clinical Trials

The first phase I safety trial for the use of bacteriophage therapy was published in the United States of America in 2009; this investigation was of the potential use of bacteriophage-based preparation in the treatment of venous leg ulcers in humans[17]. In this trial, 42 patients with chronic venous ulcers were tracked on initiation of this treatment and outcomes were measured. The study followed patients for a control and an experimental group for twenty-four weeks after therapy. The experimental group was treated topically with WPP-201, which is a bacteriophage formulation targeting *P. Aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*[17].

In this study by Rhoads et al., the proportion of healed ulcers was not significantly different between the treatment and control groups at either 12 or 24 weeks[17]. However, in this trial there was also no significant difference between them in the frequency of adverse events, in either quality or quantity. Potential limitations included the amount or type of bacteriophages used and the minute sample size, since the safety profile for the therapy did not cause any major adverse events, the study concluded that the efficacy of the product will need to be re-evaluated in a phase II study[17]. While further trials have not investigated venous leg ulcers in humans, phase I/II clinical trials have shown low to moderate efficacy in the use of this therapy for treatment of other conditions.

One such study, by Wrights et al. investigated single dose bacteriophage therapy in chronic otitis caused by MDR *P. aeruginosa*[18]. This study contained 24 patients with an illness duration of several years and were divided into a control and an experimental group. Outcome measures of clinical change such as erythema, discharge, etc., were quantified using a visual analogue scale (VAS)[18]. Bacterial levels were measured initially and at follow-up on days 7, 12 and 42 and clinical indicators improved for the phage-treated group relative to the placebo group in that study and *P. aeruginosa* counts decreased[18]. For the experimental group in this study, mean reduction of the total VAS scores at the final follow up was 50% and three patients had more than 80% reduction. In contrast, the placebo group in this study had a 20% mean reduction with no patients having more than 80% reduction. No significant changes were found in relation to audiometry and no adverse effects were reported, however, the sample size here was quite small[18]. Larger sample sizes are needed to confirm that these effects are genuine and to reduce the amount of possible errors[18].

Limitations

While the prospect of bacteriophage therapy appears lucrative, we are still a long way from bringing it into practice. Part of the reason for this is its long list of limitations, which range from our current lack of knowledge of its pharmacokinetics and pharmacodynamics to the very large variation and evolution in bacteriophages. One obstacle to employing bacteriophage therapy is antigenicity of the viruses and the immune response that follows it, which can dampen their clinical response and undermine their therapeutic value. Apart from this, we run the risk of development of bacterial strains that are resistant to bacteriophages, despite the hope that bacteriophages would evolve too

and counter this resistance. It would also be important to be vigilant of life-threatening syndromes like the reaction to endotoxin-like substances[19,20].

The literature is scant on studies with well-designed clinical trials which can evaluate the efficacy of the therapy in different patients. The studies currently published address remarkable cases where it was effective, and some indicate a safe profile for it. However, there is still little proof that this therapy can be replicated consistently in different people. It is also proving difficult to contain standardised formulations of the bacteriophages because unlike antibiotics, these are living organisms with a propensity to evolve.

Conclusion

While it is apparent that we have a long journey ahead of us for bringing bacteriophage therapy into routine practice, it is also clear that it is not just a hype. Phase 2 clinical trials are the strongest evidence so far for demonstrating the therapeutic implications of this therapy. The therapy is bactericidal when conducted accurately and there is some evidence that it can have the ability to counteract antibiotic resistance. It has also predominantly been shown to be safe and efficacious. Nonetheless, it features numerous limitations, which would have to be dealt with prior to the promise of carrying the therapy into practice. There is warranted hope that this therapy can be utilised for the clearance of infections in the future with preliminary evidence prompting further research into the area. ◄

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Declarations

The author declares no conflicts of interest.

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LITERATURE REVIEW

Adenovirus Manipulation for Use as an Effective Delivery Vector

Aidan O'Riain

Moyne Institute of Preventive Medicine, Trinity College Dublin, University of Dublin, Ireland (ORiainA@tcd.ie)

Abstract

Adenoviruses are used as delivery vectors in many different biotherapeutic systems to provide treatment options in several clinical settings. Their relative safety, potent induction of an immune response, and ease of production have allowed these vectors to appear at the forefront of clinical medicine in recent times, with applications in gene therapies, cancer treatments, and vaccines (including those for SARS-CoV-2). Their ease of genome manipulation and large gene transduction abilities make them particularly attractive for use as delivery vectors.

This paper aims to show that, despite significant challenges, adenoviruses have generally been effective as delivery vectors for gene therapies and vaccination strategies. Taking advantage of their diversity and delineated viral tropism is critical to implementing effective clinical strategies, moderating the negative effects of pre-existing immunity, combatting transient action, and optimising target cell specificity. Overall, this paper argues that adenoviral vectors are a promising tool for use in a wide range of clinical applications.

Keywords: Adenovirus, Vector, Vaccine, Biotherapeutics

Introduction

Adenoviruses (Ads) are common viruses that are non-enveloped, icosahedral, and 90–100 nm in size. Adenoviruses also contain a double-stranded DNA genome. In recent years, Ads have been developed for use as vectors to transduce genes into host cells or to induce a robust host cell immune response. Clinical applications of Ad vectors include gene therapy, cancer gene therapy, and vaccination.

Ads are suitable viral vectors due to their size and they can be easily manipulated. Using recombinant DNA techniques, manipulation is efficient as the virus can produce progeny in permissive cells, elicit high levels of protein expression, and can hold up to 38kB of foreign DNA[1]. It is possible to remove regions of the Ad genome, particularly the E1 and E3 regions, in order to make space for exogenous DNA insertion (**Figure 1**).

These recombinant viruses, carrying foreign genes, can infect a greater percentage of cells than naked DNA insertion, generating the desired population of virus-infected cells more efficiently[1].

This paper discusses the applications of Ad vectors and their limitations. Ad transgenes are delivered efficiently[2], infecting both dividing and non-dividing cells[3]. However, they only have transient gene expression[4]. This is because Ad DNA cannot be integrated into host DNA and they are immunogenic, meaning they stimulate the immune clearance of the vector[5]. Transient gene expression in Ad vectors has been observed to be maximal during the first week of expression. Despite that, no transgene expression was found at all during the twenty-one days post-administration of Ad vectors in rat cardiomyocytes[6].

Another potential disadvantage of using Ad vectors is the high levels of pre-existing immunity in humans[7,8] This pre-existing immunity is due to the seroprevalence of Ads in the population, with up to 73.1% of 1,154 subjects in a trial in China showing the presence of human Ad5-neutralising antibodies[9]. Many of us may have already been exposed and are immune to infiltration by certain Ad serotypes. In addition, despite Ads generally being regarded as safe when used as vectors, complications have arisen in past clinical trials, which will be discussed later in this paper[10,11].

Adenovirus Vectors

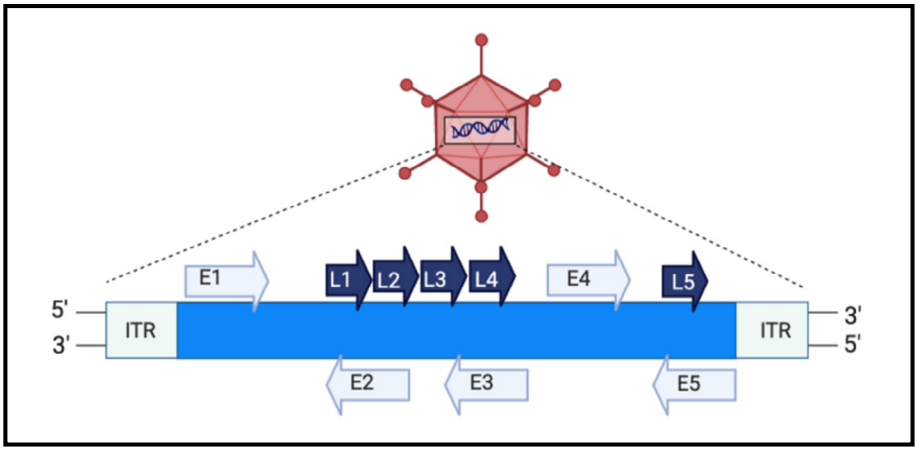
Vector Production

The production of Ad vectors is relatively easy, and high stocks of purified virus can be produced, making them attractive for clinical use[12,13]. Furthermore, they are compatible with industry-standard clinical manufacturing and thermostabilisation processes[13]. This thermostabilisation of the vector is also important for avoiding the use of cold chain technologies for storage, resulting in easier storage and improved shelf-life.

Several factors allow high titres of Ad vector production. These include their ability to be manufactured in mammalian cell cultures such as HEK293 cells, which provide trans-acting E1 proteins to allow viral replication[12]. Another factor is their stable genome, contributing to their ability to be amplified successfully. E1 proteins are essential for viral replication and early gene expression (**Figure 1**).

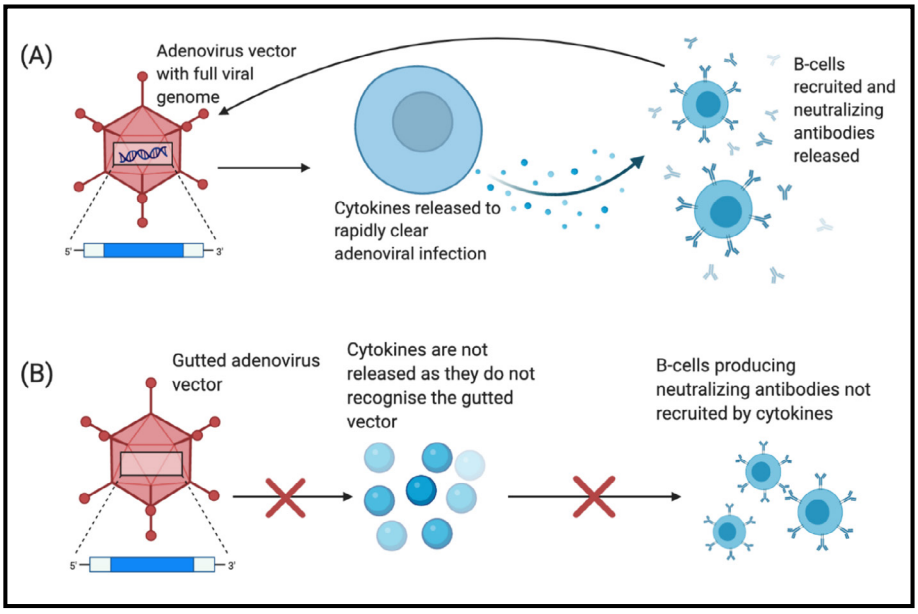
In addition to the ease of production of gene therapies and cancer therapies, studies aiming to develop vaccines against the recently emerged SARS-CoV-2 virus demonstrates that the development of Ad vectors is efficient and rapid. In terms of production, an easily reproducible murine model was developed within 2–3 weeks, that can be used to explore SARS-

Figure 1. **The Adenovirus Genome**



Adenoviruses contain a double stranded DNA genome. Early genes such as E1 are required for replication. As a result, they can be removed to render the virus replication-defective, a feature of many adenovirus vectors that contributes to the safety of their use, while expanding their inserted DNA capacity. E3 genes are also often removed to allow greater packaging space.

Figure 2. **Different Adenovirus Vector Constructions Lead to Differing Immune Cell Interactions**



A) Adenovirus vectors with full viral genomes stimulate immune cells and cytokines are released in response to the virus, leading to antibody release. B) Gutted adenovirus vectors containing only ITR regions at N and C-terminus domains can evade host immune response as their viral genome is not expressed and therefore cannot be recognized by the innate immune system to clear the virus. Thus, gutted vectors are a possible solution to the transient action of adenoviral vectors, along with pre-existing immunity to some adenovirus serotypes such as Ad5.

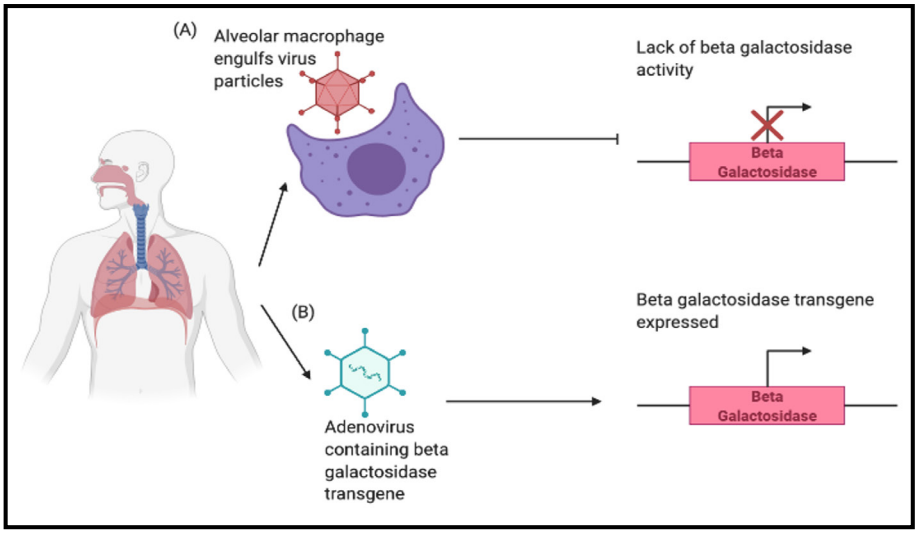
CoV-2 pathogenesis and potential vaccine strategies[14]. This method has progressed production a considerable amount in comparison to developing and breeding human ACE2-transgenic or human ACE2-knockin mice for experiments.

In some studies, using an Ad as the vector instead of an adeno-associated virus or lentivirus has been advantageous because Ad vector production does not require plasmid transfection on a grand scale. In a particular study, a single HDAd5/35++ vector stock could be used for numerous production cycles[15]. Viruses with small deletions can be propagated in cell cultures that have genetic defects to allow viral reproduction[16]. Gutless Ad vectors are defined as vectors that are manipulated to the point where it is essentially stripped of its genome, only retaining the inverted terminal repeat (ITR) regions (**Figure 2**). These gutless vectors can render the virus unrecognisable to hosts with pre-existing immunity, avoiding an anti-Ad response (**Figure 2**). However, their synthesis requires special producer cell lines[17]. In addition, gutless vectors exhibit reduced toxicity, immunogenicity, and a longer duration of transgene expression than vectors with a full or slightly removed genome (**Figure 2**)[18].

Ad vector production can be up-scaled successfully. Vectors have been scaled up to 3L bioreactors from shake-flasks[19]. Ad rabies vaccine AdRG1.3 has successfully been scaled up from 1 liter to 500 liters, while maintaining cost-effectiveness and efficacy[20]. The ability to up-scale vaccine vectors is important to maximise production and allow large doses of vaccine to be made available across the world.

Ads are easily manipulated for different treatment areas including vaccine strategies and cancer

Figure 3. **The Effect of Alveolar Macrophages on Virus Particles in the Lung**



A) Alveolar macrophages can phagocytose adenovirus particles, resulting in 70% virus particle degradation within 24 hours. B) Viral vectors containing beta galactosidase as a marker for transgene expression show that removal of these macrophages allows adenoviral gene expression.

treatments. Ad vectors were, however, originally developed as a gene therapy treatment strategy to combat cystic fibrosis (CF), with many subgroups of Ads showing an affinity for respiratory epithelia[21,22]. In fact, Ads carrying the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been used in trials[23]. However, Ad vectors struggled to treat CF patients, showing only transient expression[24,25].

Vector Administration

In terms of administration, a lot is known about intravenous (i.v.) administration. However, it is not feasible for large scale vaccination, as it has been observed to stimulate a low immune response compared to intramuscular (i.m.) injections[26]. In addition to this, i.v. administration has been observed to cause anaphylaxis. Much less is known about i.m. or intranasal (i.n.) administration, so work should continue in these areas. Following i.n. administration, humoral and cellular immune responses have been observed[27]. However, alveolar macrophages have degraded the vector in some cases. Worgall et al.[28] showed that 70% of the Ad genome in the lung was degraded after 24 hours in the presence of alveolar macrophages. When these macrophages were removed, administration of Ad vectors encoding beta galactosidase showed a substantial increase in this transgene expression, showing vector degradation was macrophage-dependent in this case (**Figure 3**).

Vector Safety

One feature of Ads that make them so suitable as delivery vectors is their ability to transport their own DNA into the nucleus[29]. Along with this, replication-incompetent Ads have been shown to act with high accuracy[30]. Since these viral DNAs that enter the host nucleus cannot integrate into the host genome, they are remarkably safe. Ad vectors are easily rendered replication-defective, as the early genes rely on E1 gene expression, so replication can be inhibited by deletion of this single E1 gene. Such vectors have been the subject of a number of safe studies in both young children and older individuals who may be deemed "at risk"[31,32].

Non-replicating vectors are particularly safe, as observed in a phase I study of an Ad4 vector vaccine for H5N1 influenza[33], and the VXA-A1.1 vaccine phase II clinical trial for H1N1 influenza[34]. Oncolytic vectors on the other hand, are vectors that are used to treat cancer by using replication-competent virus inside of cancer cells to kill them. It is important for these replicating vectors to be target cell specific, focusing solely on cancerous cells. A replicating vector has the potential to be dangerous and cause harm if not produced to target very specific cancerous signals, including initial tumour enlargement[35].

Gene Therapy

Applications

Ad vectors have been proven to have a high rate of gene transfer in vivo. The fact that they can be gutted and store a lot of foreign DNA is of huge benefit for therapeutics and is one of the main contributors to success in gene therapies. The first gene therapy vector of any type to be approved for public use was Gendicine[36]. Gendicine is a recombinant Ad used to express wildtype p53 tumour suppressor genes. It has been used to treat patients with p53 gene mutations in cancer treatment[37].

An example of gene therapy using viral vectors is the delivery of the Cas9 gene and guide RNA (gRNA) to host cells to treat Duchenne muscular dystrophy (DMD)[38]. This study by Boucher et al. showed that multiple routes of gene insertion could be achieved using the flexibility of Ads[39]. The Cas9 and gRNA alone could induce gene editing via non-homologous end joining (NHEJ), while the use of a second vector containing homology directed repair (HDR) template DNA could allow the HDR pathway to be used[39]. NHEJ is prone to unwanted nucleotide deletion errors but is very efficient[39]. Alternatively, the HDR pathway was less efficient but was more precise in its insertion[39]. This Cas9 gene remedied DMD by causing

exon skipping in the host cell nucleus of the dystrophin gene[40].

Issues

Clinical risks. Gene therapies can be very effective but have some potential underlying issues. Ad vectors have been under public scrutiny after the death of Jesse Gelsinger in 1999. This death occurred after clinical trials aiming to treat ornithine transcarbamylase deficiency resulted in a cytokine storm, leading to multiple organ failure and, in turn, the death of Gelsinger[11]. This hampered viral vector development and threatened public faith in gene therapy, drawing specific criticisms of the 38 trillion particle dose of Ad the patient had received[10].

Tropism. At a molecular level, issues can also arise. Vector tropism—especially to the coxsackievirus and Ad receptor (CAR)—can be affected by low expression of CAR on the surface of target cancer cells and haematopoietic stem cells[41]. To solve this problem, studies are looking at the modification of Ad fibre domains to alter its tropism (**Figure 4**). This can be done through pseudotyping (replacing the entire fibre or knob domain with that of a different Ad serotype) or xenotyping (adding fibre elements of Ad serotypes that are nonhuman) the vector fibres[38].

The fibre proteins of Ads are the main determinant for their tropism. Two amino acid mutations in the AB loop of the Ad serotype 5 fibre knob showed reduced liver tropism, accompanied by increased gene transfer in low-CAR or CAR deficient cells[42]. Altered tropism was initially shown when Ad5 fibres were replaced with Ad7 fibres. The altered chimeric fibre knobs resulted in a difference in tropism in host cell binding (**Figure 4**)[43].

Liver tropism that is often seen in Ad vectors can damage their efficiency and ability to reach target cells elsewhere in the body. Recent studies have been focused on retargeting vectors through modifications of this discussed fibre domain. The fibre shaft contains a KKTK amino acid region that binds to coagulation factors and heparin sulfate molecules on the cell, found in abundance on liver cells. To combat this, studies have mutated this KKTK region to reduce liver tropism and increase gene transfer to target cells[44].

Despite Ad vector tropism modifications being limited today, some breakthroughs have been made. Even with transient expression, Ad5 vectors with recombinant Ad37 fibre knobs transduced NK-92 natural killer-derived cells to cancerous cells more efficiently than native Ad5 vectors[45]. This demonstrates the power of chimeric fibre knobs as Ad5 vectors often show not only transient expression, but much of the population has pre-existing immunity to this serotype.

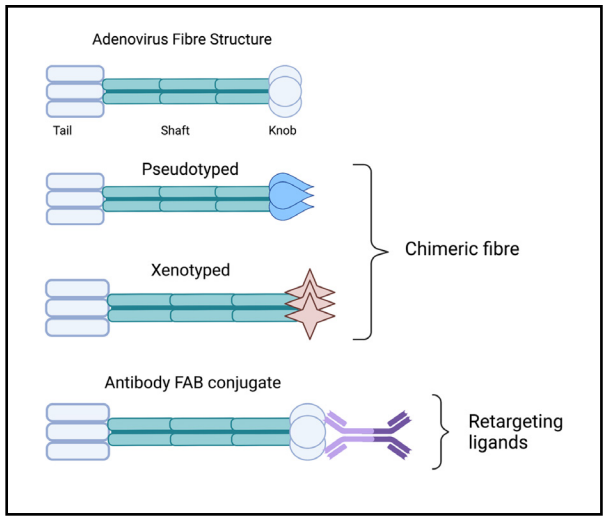
Cancer Gene Therapy

Applications

Ad vectors have been a focus of directed cancer treatments for specific human cancers. Gendicine was one such drug designed to treat cancer. In contrast to the replication-defective Ads being used in gene therapies and vaccines, cancer therapy uses replication-competent viruses. This strategy is used as it allows the viruses to lyse cancer cells through the lytic life cycle of the virus.

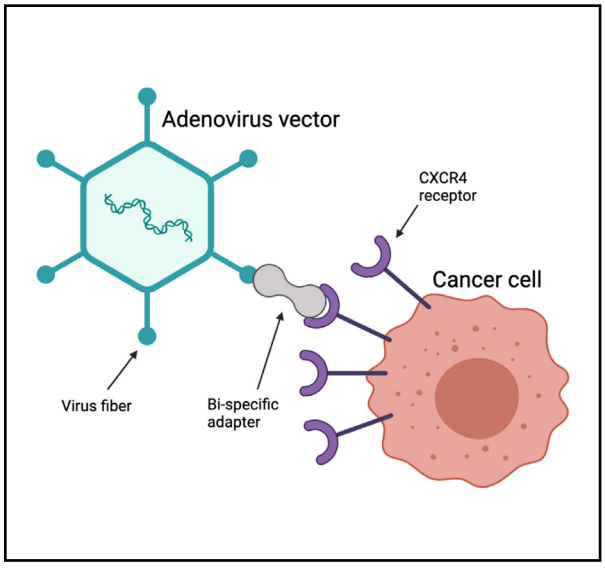
These vectors can have cancer-specific promoters

Figure 4. **Adenovirus Fibre Domain Structures**



Adenovirus fibre domains can be manipulated and modified in a number of ways to alter their receptor specificity to focus on target cells more efficiently. Most modifications take place in the fibre knob domain. Chimeric fibres alter virus tropism while retargeting ligands (adapters) physically link virus particles to host receptors such as CD40L. Peptide insertions (not shown) can alter fibre properties too.

Figure 5. **The Cellular Action of Bispecific Adapters in Retargeting Viral Vectors**



Adenovirus fibres can be retargeted with a tropism for cancer cells using the bispecific adapter sCAR-CXCL12. This retargeting allows the vector to bind the overexpressed CXCR4 chemokine receptors found on cancer cells.

that replace the E1A enhancer/promoter and/or the E4 promoter[36]. ONYX-015 was the first oncolytic Ad vector to be examined in clinical trials. It lacked the E1B-55K protein[46]. This E1 gene deletion in Ad vectors can be replaced with other genes that stimulate an immune response in only p53-deficient target cells. ONYX-015 targets cancer cells with different late RNA export mechanisms, rather than p53 inactivation[47]. ONYX-015 treats cancer cells using different mechanisms to chemotherapy, so it shows good potential in patients that have not responded to chemotherapeutic treatment[35]. There have been similar studies on E1-replaced vectors. For example, when the E1A promoter in the oncolytic CV706 Ad vector was replaced by the prostate-specific antigen (PSA) promoter-enhancer[48], CV706 demonstrated cancer-cell–specific tropism, selectively targeting and killing prostate cancer cells.

In general, cancer gene therapies use replication-competent Ads to lyse cells. Techniques used by Boucher et al.[38] have shown high-level selectivity in the delivery of CRISPR-Cas9 technology to mutated oncogenes by Ads, causing knockout mutants or inhibition. CRISPR-Cas9 deletions have reduced tumour growth in mouse lung cancer xenograft models using knockouts of L858R mutations in the EGFR-overexpressing lung cancers[30]. In some cancer treatments, the gene knockouts may be in the vector itself. The removal of E1B 19kDa is important to allow anti-tumour effects of p53-induced apoptosis in cancer cells[49].

Different approaches and techniques have been explored to combat cancer using viral vectors. The cancer gene therapy drug enadenotucirev was the first oncolytic Ad to be successfully designed using the directed evolution approach[50]. This approach aims to simulate natural selection using genetic diversification followed by phenotypic selection[50]. Enadenotucirev stimulates pro-inflammatory immune responses that stimulate an anticancer response. This is achieved through its transgenes expressing CD40 agonists, interferon-alpha, and chemokines CXCL9 and CXCL10, which promote a pro-inflammatory response. Enadenotucirev has a dual mechanism of action and including stimulating immune response, it binds CD46 or desmoglein 2,6, both of which are often found on carcinoma cells, causing ischemic cell death through ATP depletion[51]. Enadenotucirev has been trialed in concert with chemoradiotherapy to act in locally advanced rectal cancers, utilising its selective toxicity in carcinoma cells[52].

Issues

Tumour enlargement. These oncolytic Ad vectors have encountered issues in their progression to treat cancers. Reid et al.[35] found that transient enlargement of tumours was discovered in some patients, resulting in their removal from the clinical trial. It has been suggested that the inflammatory response as a result of viral sensing in the host may have led to tumour enlargement.

Tropism. Accompanying such limitations, the low expression of CAR receptor on cancer cells has been a treatment barrier. As discussed above, pseudotyping the Ad fibre is being attempted **(Figure 4)**[38]. An example of this occurring is expanding tropism to hematopoietic stem cells using Ad35 fibre targeting CD34+ cells, which binds to these cells independently of integrins[53]. Another technique that can be used is chimeric adapter proteins **(Figure 5)**. These proteins contain binding domains for the Ad fibre knob proteins and a binding domain for the cells of interest. CXCR4 chemokine receptors are upregulated in many cancer cells. As such, a bispecific adapter chemokine, sCAR-CXCL12 was developed to retarget Ad vectors to these receptors on specific cancer cells—particularly in breast cancer and melanoma—through direct interactions **(Figure 5)**[54].

Overcoming tropism specificity issues has become an increasing problem in Ad vector cancer treatment. Hexon interactions with coagulation factors (FX) have been hypothesised to be a major player in hepatocyte transduction by systemic delivery of Ads[2]. Different Ad serotypes have shown a range of binding affinities for coagulation factors. Some bind with high affinity, while other serotypes such as subgroup D do not bind to FX at all[55].

Vaccines

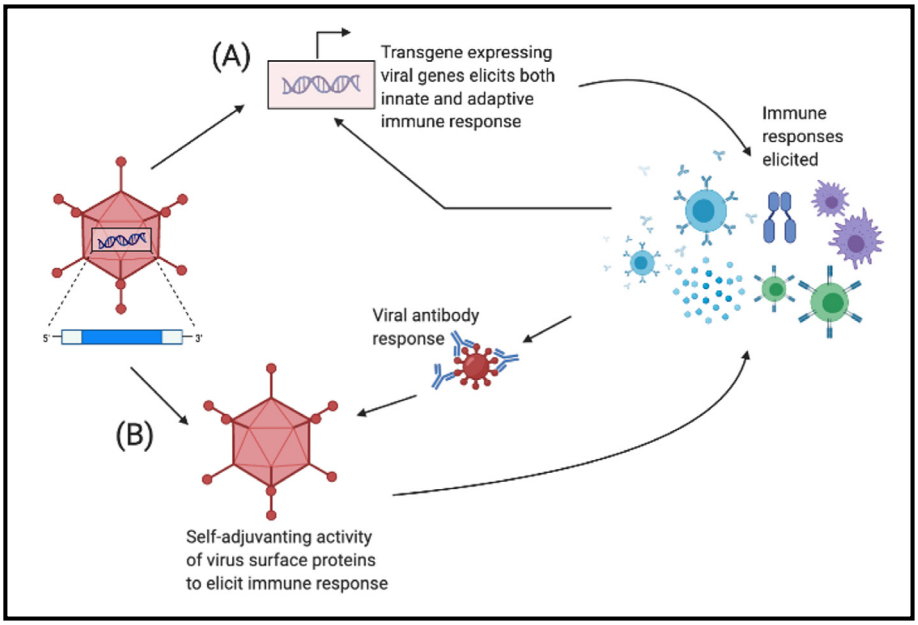
Applications

Ad vectors have increasingly been researched for use as vaccines, most recently in the fight against SARS-CoV-2. The Oxford/AstraZeneca ChAdOx1 vaccine, using a chimpanzee Ad, has a reported 62.1% efficacy[56]. This vector encodes the full spike protein of SARS-CoV-2[57], and the use of non-human Ads avoids pre-existing immunity. Similarly, the single dose Ad26 SARS-CoV-2 vector vaccine developed by Johnson & Johnson has completed phase 3 trials and has been licensed, distributed and administered worldwide[58]. Ad26 is a human Ad with strikingly low seroprevalence, yet another strategy to combat the threat of pre-existing immunity.

As of the writing of this paper, 9 of 27 vaccine candidates are currently in clinical trials to treat SARS-CoV-2 use viral vectors[59]. In many of these candidates, including the ChAdOx1 nCoV-19 vaccine, immunoglobulin G (IgG) response was higher in patients that received prime-boosted vaccine than in those who did not[59]. The immune response acted against the spike proteins within 28 days and showed both cellular and humoral immunity. Previous attempts to increase the immunogenicity of ChAdOx1 nCoV-19 saw rAd5 fibre and penton RGD motifs added. However, this did not increase the vaccine's immunogenicity[60]. Interestingly, a weaker first dose was given to some trial participants, conferring a higher efficacy (90.0%) than two full dose measures[56]. This shows that a high number of factors must trigger immune response when using vectors. This vaccine is very promising as it elicits a notable immune response in an older age group. Other viral vector vaccines in clinical trials have either shown reduced immunogenicity in older groups or have not yet been tested in these groups[61].

Other trials are underway regarding the use of a non-replicating type 5 Ad vectored SARS-CoV-2 vaccine[62]. The aim here was to express the entire spike

Figure 6. **Adenovirus Vector Vaccination**



Vaccination using adenovirus vectors can elicit immune response in two complimentary ways. A) Using transgene expression to allow access to host cells and elicit both innate and adaptive immune responses. B) The adenovirus itself, when at low seroprevalence, can have self-adjuvating properties through antiviral host antibody response to clear the adenoviral infection.

gene of SARS-CoV-2 to induce an immune response. Significant humoral and cellular immune responses were observed within 28 days, particularly in the younger populations[62].

In the case of SARS-CoV-2 vaccines, it is undeniable that Ad vectors have some advantages over other strategies. The main advantages are, while maintaining their efficacy, they have a low cost and an ability to be stored at regular refrigerator temperature. In contrast, the mRNA vaccine candidates must be stored at -20°C (Moderna) and -80°C (Pfizer-BioNTech)[63]. In addition, using human Ad vector technology is extremely cost efficient. For example, the European Union (EU) has paid 2.15 USD per dose of AstraZeneca ChAdOx1 vaccine while the Pfizer-BioNTech mRNA vaccine costs the EU 14.70 USD/dose[64]. These properties may prove to be of huge importance in facilitating mass vaccination by simplifying the logistics behind distribution and storage.

Along with delivering transgenes effectively to stimulate a strong humoral and cellular immune response, Ad vectors can behave in an adjuvant-like fashion, stimulating the immune system through toll-like receptor (TLR)-dependent and independent pathways[65]. TLRs are proteins that recognised conserved molecules in microbes and stimulate the innate immune response as a result. These pathogen recognition receptors (PRRs) recognise the virus and stimulate the host immune response to clear the virus. The vectors are attractive candidates for vaccines as they induce potent inflammatory responses after vaccination, both innate and adaptive, as shown in **Figure 6**. They can be enhanced using specific targeting such as having the Ad vector vaccine itself target dendritic cells[66]. Similar to the fibre changes in many gene therapies, Ad5 fibre is genetically modified to express hCD40L using FAB antibody conjugates (**Figure 4**), which in turn is what targets dendritic cells in this study by Sharma et al[66].

Vector vaccines have also come to the forefront of public health in recent years in the form of Zika virus vaccine candidates. The Ad vector vaccine ZIKV, uses Ad4-prM-E. One of the main advantages with this virus is its low seroprevalence, leading to anti-ZIKV T-cell response without eliciting many anti-ZIKV antibodies (**Figure 6**)[67]. The interesting aspect to this response was that it was a result of the serotype of Ad, not the transgene (**Figure 6B**). The same result was found using this vector to vaccinate against influenza hemagglutinin (HA) in this study. The study conducted by Bullard et al.[67] showed how Ads self-adjuvant properties can be utilised to create vaccines, almost irrespective of transgene effects (**Figure 6B**). Ad4 has been shown—due to its low seroprevalence—to be useful as a vaccine. It has been shown to induce anti-H1N1 immunity against influenza and was observed that Ad4 provided far superior protection in mice compared to Ad7 vectored vaccine strategies[68]. However, a major issue is the study lacks information on HA inhibition or virus neutralisation.

Issues

Clinical risks. The SARS-CoV-2 vaccine development path has not all been clear. The Sputnik V vaccine that uses an initial dose of Ad5 vector and a second dose of Ad26 vector has reported around a 91.6% efficacy[69], despite claims that their results are more compatible with an efficacy of around 60%[70]. The differences in reported efficacy appear to be a result of political controversy and the way in which the trials were conducted and published. Additionally, this vaccine is under scrutiny for its approval for use, as phase 3 of the clinical trial was still ongoing at the time of administration in Russia. Questions have been raised by immunologists about the efficacy of the two full-dose ChAdOx1 vaccines. Their

concerns are that a 62.1% efficacy is not high enough to confer herd immunity, along with adverse effects such as blood clotting being noted to be caused by this ChAdOx1 vaccine[71].

Ad vector vaccines against other viral infections have encountered development issues. Merck completed a test-of-concept study (STEP study) on the development of a MRKAd5 HIV-1 gag/pol/nef vaccine. Surprisingly, this replication-deficient Ad vector vaccine showed higher HIV-1 incidence than those who were treated with placebo[72]. What was completely unexpected was that those who had high titres of antibodies against Ads showed an increased incidence of HIV infection. The authors suggest a possible reason for this is that antibody and virus presence may lead to T cell activation, providing an environment that facilitates HIV infection[73].

Pre-existing immunity. A challenge to vaccination using Ad vectors is that the human population may have pre-existing immunity to specific Ad serotypes, preventing widespread protection. However, it is also worth noting that Ad vector vaccines have been shown to be able to overcome possible pre-existing immunity by increasing the dosage of the vaccine or using a different route of vaccination. Sayedahmed et al.[74] showed that along with the ability to overcome immunity, annual vaccines would be feasible. The challenges of pre-existing immunity to certain Ad serotypes in the human population have previously been outlined and can have hampering effects on their clinical use. Components of the immune system such as neutralizing antibodies and Ad-specific T cells can dampen the effects of Ad vectors in individuals with pre-existing immunity[75].

Overcoming pre-existing immunity. Sayedahmed et al.[73] showed that in mice, immunity levels decreased over time, with similar protection levels to mice with no immunity 10 months post-vaccination. What appears to be key is the time between vaccinations, allowing degradation of immunity. So, this pre-existing immunity may be seen to wane over time, allowing successful vaccination if apt time is allowed between vaccinations. However, this is yet to be studied in humans. The Ebola virus (EBOV) has caused many problems in Africa in recent years, similar to the Zika virus previously discussed. EBOV vaccine studies have used similar approaches to SARS-CoV-2 strategies, with both using viral surface proteins to elicit an immune response. In this case, EBOV glycoproteins are expressed as a transgene within an Ad26 viral vector[76]. Pre-existing immunity was very low in human trial participants (3.4%). In non-human primates, follow-up boost vaccines of Ad35 were given in response to EBOV glycoproteins[77]. This Ad35 boost provided a reasoning behind how immunity was bypassed, proving to be superior to Ad5 in binding dendritic cells. Additionally, Ad35 has a natural tropism for diverse primary human cell types[78]. Geisbert et al.[77] showed another interesting feature of Ad vectors in that they can be manipulated depending on administration techniques to confer either immediate immunity or longer lasting immunity. This may also diversify the possibilities of Ad vectors as vaccines, depending on whether emergency immediate immunity is required or a longer lasting protective cover is needed.

Conclusion

Based on the literature, Ads as delivery vectors have generally been effective for gene therapies and vaccination strategies. Despite setbacks in vector development and gaining public trust post-Jesse Gelsinger's death and the Merck STEP study, there is most certainly a future for Ad vector therapeutics. For example, the ChAdOx1 SARS-CoV-2 vaccine is immensely important (apart from their obvious immunogenicity) in providing a cost-efficient and accessible vaccine.

Ad diversity and delineated viral tropism is key to their success as vectors. Future studies of Ad vectors should look to develop these differing tropisms through complexes of different subtypes to adapt to their host, providing efficient transgene delivery and/or eliciting an immune response. These mosaic vectors help to overcome the issue of pre-existing immunity, while gutless vectors can also be used to avoid this anti-Ad immunity. Heterologous vectors using prime–boost strategies have also yielded successful results but require further development.

It is clear that studies should not become focused on specific Ad serotypes. If a single serotype was solely used in gene therapies and vaccination, immunity would be quickly developed by the global population. As a result of host adaptation, the library of Ads needs to include many chimeric surface proteins and mutated genotypes to allow for a broad range of possible treatment options. Pre-existing immunity is unavoidable but requires attention as Ads are encountered often in everyday settings, especially being implicated to sometimes cause the common cold[79,80].

In vaccination and cancer gene therapy, immunity must be managed. Vector transduction and its immunogenicity may be designed to elicit an immune response and act as a self-adjuvant. However, striking a balance of immune response—weak enough so the vector is not cleared too rapidly, while also strong enough to sufficiently stimulate adaptive immune memory cells—remains difficult. Evidence of this correct balance of induced immune response has been observed in the use of non-human and low seroprevalent Ad vectors.

Ad vectors that deliver CRISPR/Cas9 systems to target cells have shown promising results and could work to improve the efficiency and effectiveness of gene therapy using viral vector delivery. In gene therapy, it is important to be wary of possible vector contamination when using helper Ads, and to note that transient vector activity may arise.

Ad vectors demonstrate an encouraging source of gene delivery and bring about a new era of therapeutics. They show huge benefits when applied successfully, despite their many pitfalls. If these various pitfalls discussed (such as pre-existing immunity, transient action, exceedingly robust immune response, and target cell specificity) can be overcome, the potential of these delivery platforms is immense. Ad vector delivery could be transformative for therapeutic molecular biology and, indeed, for global human health. ◄

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Declarations

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LITERATURE REVIEW

The Trojan Bacillus: Transgenic Bacteria in Cancer Therapy

Matthew Thomas[1]

[1] *School of Biochemistry and Immunology, School of Medicine, Trinity College Dublin, University of Dublin, Ireland (MaThomas@tcd.ie)*

Abstract

A classic conundrum in oncology is the identification of cancer-specific, druggable molecules which can be targeted with minimal systemic toxicity. A novel candidate for administering cancer therapeutics has emerged in bacteria, which may prove to be excellent delivery vehicles for biologics whose systemic delivery causes severe and unacceptable adverse effects. Bacteria are well-suited to this role due to their ability to colonise tumour microenvironments, synthesise drug molecules, and potentiate innate and adaptive immune responses. Genetic clockwork in the form of quorum sensing mechanisms allows these bacteria to lyse on demand, releasing therapeutic payloads into tumours. Recent in vivo evidence outlined here support this hypothesis, yet there is a great deal of research and refinement still to be done.

Keywords: Synthetic biology, Oncology, Targeted therapy

Introduction

In the late nineteenth century, American surgeon Dr. William Coley noted that injection of bacteria directly into sarcoma induced a number of remissions, some permanent[1]. Recently, the use of bacteria as delivery systems for immunotherapy biologics has emerged. The advantages of bacterial delivery systems are numerous. Bacteria may localise to tumour microenvironments autonomously via chemotactic receptors and release therapeutic biologics within tumours, limiting systemic toxicities. Bacteria can sense population growth and lyse *en masse* through engineered lysis circuits. Furthermore, they can be controlled by external signals while being engineered to produce a range of therapeutic molecules. Such advantages are reviewed elsewhere[2].

Several murine assays demonstrate the efficacy of bacterial delivery of anti-phagocytic and immune checkpoint inhibitory biologic drugs, each inducing tumour remission and affecting systemic immunity, thus demonstrating proof of concept for intratumoural delivery of modified bacteria[3,4]. Further evidence and refinement is required before this can be tested in humans, however. This review will examine the role of lysis circuits in localising bacterial colonisation of tumours, the benefits, and limitations of bacterial drug delivery in cancers, focusing on the drugs which can be delivered, and current in vivo evidence of bacterial drug delivery systems in murine cancer models.

Lysis Circuits

Orchestrating Bacteria: Quorum Sensing

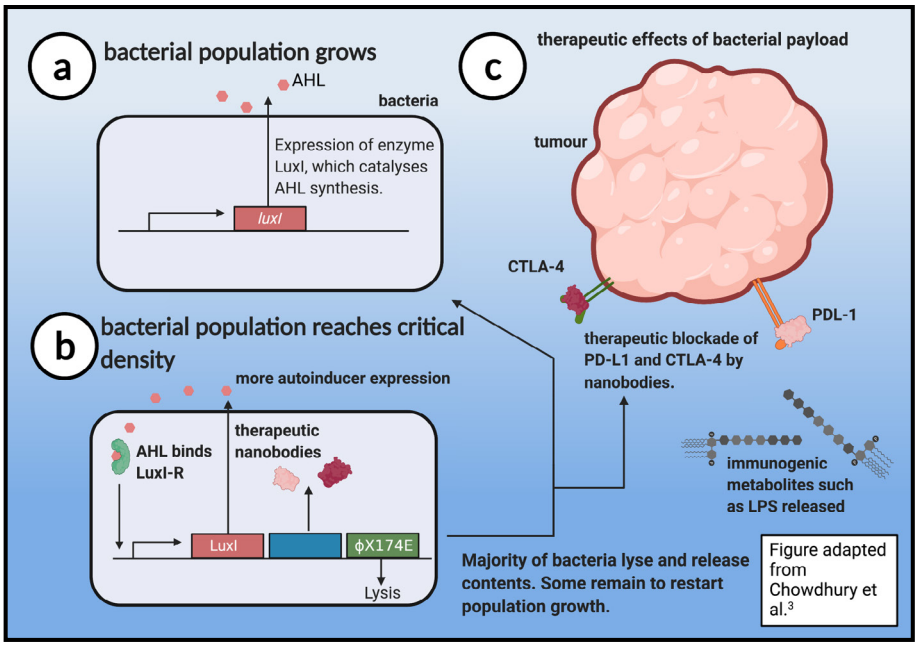
Bacteria functioning alone is both costly and likely ineffective in numerous instances, and consequently bacterial populations coordinate certain activities on a population wide basis, in a system called quorum sensing. This system allows for modulation of gene expression based on the population density of a bacterial population, with each individual simultaneously releasing and sensing a signal molecule, called the autoinducer. Given that these autoinducers are constitutively expressed, their concentrations rise in a stepwise manner with population density[5]. The behaviours controlled by quorum sensing would be ineffectual if undertaken by a single bacterium, and therefore must be coordinated among a larger population of bacteria.

Wild type bacteria use quorum sensing to affect bioluminescence, virulence factor production, biofilm formation and the uptake of DNA[6]. In gram-negative bacteria, the autoinducers are generally acyl-homoserine lactones, which diffuse freely across bacterial cell membranes, activating receptors present either on the plasma membrane or free in the cytosol of neighbouring bacteria. Receptor binding induces gene expression underpinning the aforementioned functions, as well as the expression of the autoinducer resulting in feed forward signalling. Thus, gene expression across individual-bacteria can be coordinated in concert upon the bacterial population reaching a critical population size[7]. It is precisely these characteristics of quorum sensing; that it acts on whole populations of bacteria and affects altered gene expression, that it holds value in bacterial drug delivery. Quorum sensing can induce bacterial drug synthesis, release, and population containment all through expression of transfected genes, whose expression is coordinated among the bacterial population.

How to Control a Bacteria: Synchronised Lysis Circuits

Therefore, we need not rely on natural quorum sensing

Figure 1. **Bacterial Circuitry in Action (Adapted from Chowdhury et al.[3])**



The diagram illustrates the operation of a Synchronised lysis circuit: A) Constitutively expressed autoinducer concentration increases with population density. B) Autoinducer/receptor interaction results in expression of autoinducer, therapeutic payload and lysis protein. C) Therapeutic payload released into tumour microenvironment with concurrent immune activation via release of bacterial metabolites.

mechanisms as we can modify existing ones to generate more efficient bacterial drug delivery systems. This was first demonstrated by Danino et al.[8], with their simple circuit consisting of three genes flanked by identical upstream promoter regions, *luxI*. The first gene codes for LuxI, which catalyses acyl-homoserine lactone (AHL) synthesis. AHL diffuses extracellularly and binds to the constitutively expressed intracellular receptors, LuxI-R. Once bound, these bind the luxI promoter region thus making a positive feedback loop-potentiating more AHL synthesis, and consequently more receptor binding and consequent gene expression (**Figure 1**).

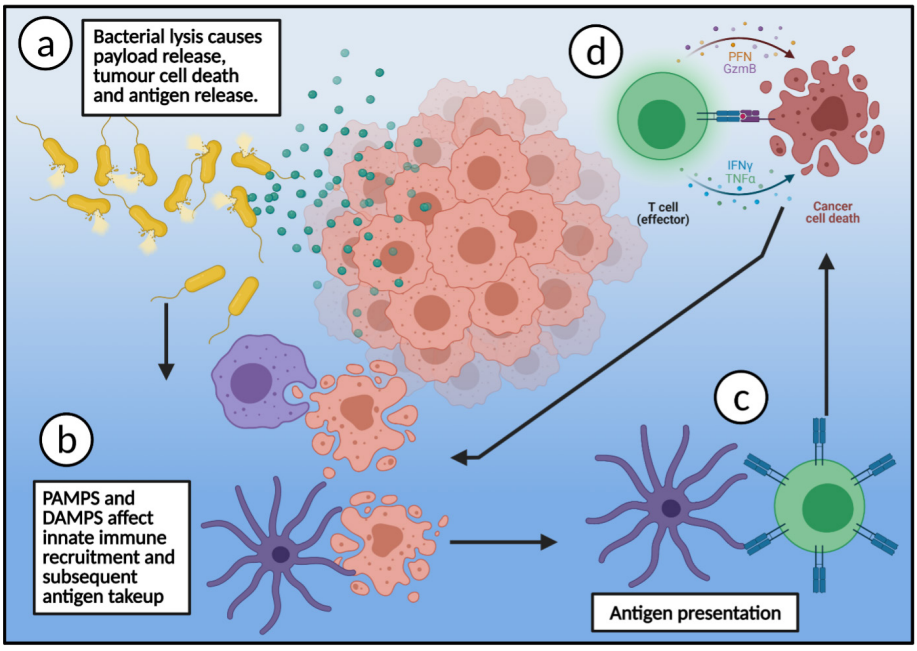
Moreover, this complex also induces the expression of the gene *AiiA*, which forms a negative feedback loop by catalysing acyl-homoserine lactone degradation. Finally, AHL-LuxI-R dimers induce the expression of the reporter gene, green fluorescent protein (GFP). These authors noted periodic oscillations in fluorescence, demonstrating increasing and decreasing GFP expression[8]. Thus, these three genes allowed for population-wide changes in gene expression, showing successful establishment of a genetic circuit constituting artificial quorum sensing. In subsequent assays, GFP is replaced by bacteriophage lysis genes. Benefits of synchronised bacterial lysis in cancer therapy are threefold. Firstly, release of bacterial cytoplasmic and membrane material into the tumour microenvironment potentiates pattern recognition receptor signalling, innate immune activation, and antigen presentation thus driving a cycle of progressive anti-tumour immunity as adaptive immune cells drive tumour cell killing and further antigen release. Secondly, quorum sensing circuits may include genes for anti-tumour biologics, that are released into the tumour as the bacteria lyse. Lastly, mass lysis of the bacterial population limits its size and prevents systemic toxicity from excessive release of bacterial pathogen-associated molecular patterns (PAMPs) (**Figure 2**).

Engineered groups of genes that are based on quorum sensing and which result in bacterial lysis are called synchronised lysis circuits (SLC). Such circuits are similar in composition to the previously mentioned oscillatory circuit, but carry genes for drug production as well as lysis, both of which contain the *luxI* promoter. The phage lysis gene φX174E is used in such circuits, and several different payloads can be expressed by the transgenic bacteria (**Figure 1**). An example is haemolysin E, a pore forming anti-tumour toxin. Din et al., used such a system to demonstrate the necessity of the SLC by incubating HeLa cells with haemolysin E expressing *Escherichia coli* (*E. Coli*) strains either with or without a SLC[9]. The SLC+ stain induced almost total loss of viability in these cells, whereas the bacteria without a SLC only induced a small rise in nonviability, thus demonstrating the efficient delivery of therapeutic payloads allowed for by SLCs[9]. While this result was seen in cell cultures, further in vivo evidence of SLC efficacy is outlined below.

Drug Synthesis

Having examined quorum lysis, its immunostimulatory effects and its benefit in drug release, our attention must now turn to bacterial synthesis of immunotherapeutic or oncolytic agents. Of particular interest is the bacterial expression of nanobodies. These are camelid-single domain immunoglobulins, whose expression by programmable bacteria is advantageous for several reasons. For one, while numerous licensed cancer therapies consist in monoclonal antibodies, these are not readily expressible in bacterial vectors, particularly due to lack of bacterial glycosylation systems. Despite this, IgG antibodies lacking glycosylation have been

Figure 2. **The Cycle of Antigen Release, Presentation and Effector Response**



A) Release of bacterial payload directly induces tumour cell death. B) PAMPs released by the bacteria, and damage-associated molecular patterns (DAMPs) released by tumour cells recruit immune cells like macrophages and dendritic cells that phagocytose tumour antigens and migrate via afferent lymphatics to local lymphoid organs. C) Antigens are presented to naïve T-cells, thus activating and mounting of effector response. D) Effector T-cells induce tumour cell death and antigen release, beginning the cycle again.

expressed in *E. coli*, although not in high yield and crucially, not correctly folded[10]. Thus, while bacteria have several advantages as drug delivery systems, this will likely not extend to delivery of monoclonal antibodies, which need eukaryotic expression systems to function properly. If we want bacteria to synthesise therapeutic compounds within the tumour microenvironment, we must look beyond monoclonal antibodies.

These challenges are not present in the expression of nanobodies, however. Moreover, the large size of antibodies, at 150kDa, restricts their access to tumour antigens with only 20% of administered monoclonal antibodies, eliciting their desired effects. Nanobodies sidestep this by virtue of their relatively small size allowing for greater tumour penetration[11]. This smaller size renders conventional infusion difficult however, as nanobodies are rapidly cleared by glomerular filtration, necessitation high dosing frequency[12]. This, however, may not apply in SLC+ bacterial delivery vectors, where expression of the nanobody occurs autonomously and continuously. In this context, rapid nanobody clearance is advantageous, abrogating excessive nanobody accumulation and resulting systemic toxicities.

Examples of therapeutic nanobodies, expressible in bacteria systems and efficacious in cancer, are those directed against immune checkpoints. Immune checkpoints are inhibitory signals that regulate the action of lymphocytes and are regularly exploited by cancers as a means of evading immunosurveillance. The most notable of these checkpoints are cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death ligand-1 (PDL-1), and PDL-1 receptor PD-1. These are all targeted by the FDA approved monoclonal antibodies, for instance Ipilimumab and Pembrolizumab, and have been proven efficacious in several cancers and are currently being investigated by numerous clinical trials for a wide range of indications. Mutation burden in cancer increases with time, and this results in truncated or otherwise altered proteins expressed at the plasma membrane. These are referred to as neoantigens, and because the immune system was not tolerised to them during thymic selection, it is possible to mount effective anti-tumour immune responses against them. Indeed, neoantigen burden is a predictive marker in checkpoint inhibitor treatment and perhaps will be beneficial in selecting patients in which bacterial delivery of such interventions will be of most benefit[13].

Proof of Concept

Immune Checkpoint Inhibitor Delivery

Drugs targeting CTLA-4 and PDL-1 are efficacious individually but have been proven to be of greater benefit in combination than as monotherapies[14]. Despite the benefit in efficacy, severe toxicities are observed in combination regimens. Moreover, the majority of patients that discontinued combination therapy in one trial had seen objective benefit, but the off-target effects were such that monotherapy was favoured[15]. Owing to these severe off-target effects, more targeted delivery systems are required to maximise efficacy with concurrent reduction in harm. This has been demonstrated in murine models using programmable, non-pathogenic bacteria. Gurbatri et al.[4] infected mice with A20 cells, a murine model of sarcoma that has shown response in past experiments to anti CTLA-4 and PD-L1 agents. In this study, these mice developed tumours in their hind flank and were treated with bacteria expressing a SLC and two nanobodies, each targeting either PDL-1 or CTLA-4.

When this strain was compared against a control with multiple intratumoural injections of bacteria,

significant therapeutic effects were observed with tumours partially or fully regressing[4]. Furthermore, the study showed that there was an increased survival benefit and no visible liver metastasis. These results were highly statistically significant (P<0.0001). In tumours treated with the programmed bacteria, flow cytometry demonstrated increased infiltration by CD8+ T-cells and proliferation of CD4+[4]. Further, the necessity of the SLC was demonstrated by comparing regression in mice treated with SLC+ and checkpoint inhibitor expressing bacteria, SLC- bacteria and lysate from SLC+ bacteria, which again showed significant results (P<0.0001)[4], illustrating that therapeutic payload as well as lysis circuits are required for effective induction of cancer remission.

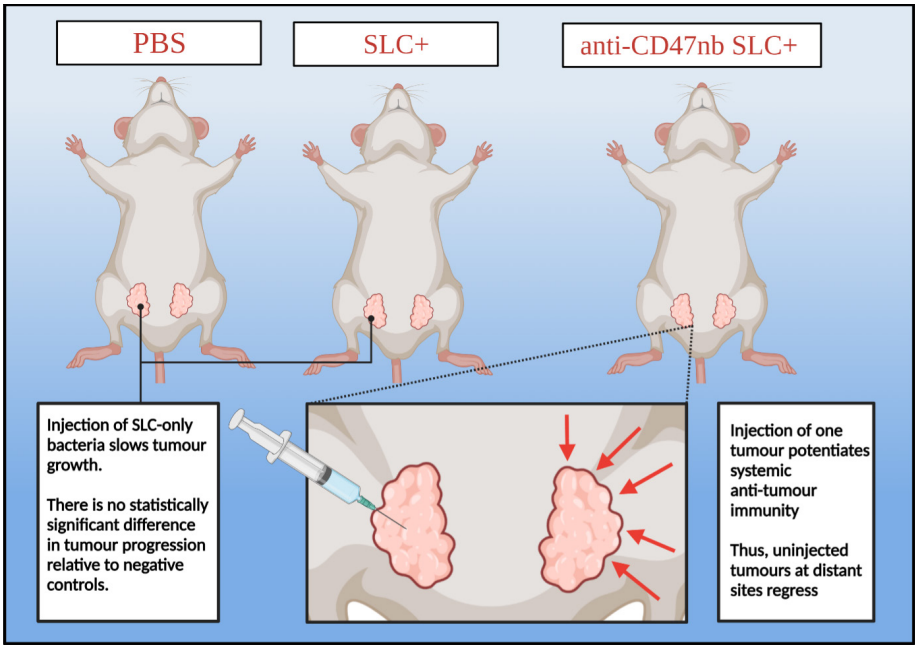
Furthermore, a systemic immune response was seen, with non-treated tumours regressing in mice with one other treated tumour. Finally, two weeks after administration, serum samples taken from treated mice showed no increased titre of TNF, demonstrating the lack of systemic inflammation. The authors attribute this lack of pathological systemic inflammation to the SLC containing the bacteria, and the success to the constitutive expression of nanobodies intratumourally[4]. Additionally, it is likely that the nanobodies used in this assay were being more readily cleared from the blood, therefore they were less likely to cause off target toxicities, further emphasising the potential benefits of this delivery system. In summary, intratumoural delivery of SLC+ bacteria that expressed checkpoint inhibitory nanobodies affected regression of the infected tumour as well as distant tumours, demonstrating these bacteria potentiated both local and systemic anti-tumour immunity. This occurred in the absence of systemic inflammation, bolstering the notion that SLCs maintain the bacterial population with the confines of the tumour microenvironment.

Anti-CD47 Delivery

Another in vivo assay evaluating bacterial delivery of immunotherapeutics was carried out by Chowdhury et al.[3], investigating nanobody mediated blockade of tumour-expressed CD47. CD47 is an antiphagocytic cell surface receptor understood to be expressed in numerous human malignancies. The authors noted however, that while blockade of CD47 does increase tumour cell phagocytosis and antigen cross presentation in murine models, it also caused anaemia and thrombocytopaenia in human trials[3]. As such, the rationale underlying this experiment was to localise CD47 blockade to avoid such systemic toxicities. The authors developed an *E. coli* strain expressing an SLC and a CD47 targeting nanobody. Balb/c mice were infected with A20 lymphoma cells on both hind flanks and subsequently were treated with either phosphate-buffered saline (PBS), SLC *E. coli*, or SLC *E. coli* expressing CD47 nanobody[3]. Initially, the cohort treated with SLC+ bacteria showed slowing of tumour growth, owing likely to the release of bacterial PAMPs and thus innate immune activation. Ultimately, tumour progression in these mice showed no statistically significant difference to the PBS treated tumours[3] **(Figure 3)**.

By contrast, the nanobody treated group (anti-CD47nb SLC+) showed marked clearance of established A20 tumours. Unlike the other groups, these animals rarely developed liver metastases. 80% of these cohort survived more than 90 days and these animals did not develop tumours when rechallenged by injection of A20 cells, as opposed to the naïve mice which developed

Figure 3. **Summary of Chowdhury et al. (2019)[3]**



While SLC+ bacteria slow tumour progression, this is not statistically significantly greater than PBS-treated tumours. In contrast, anti-CD47nb SLC+ bacteria elicit regression in the tumour into which they are injected, as well as distant tumours and tumours established after bacteria injection.

tumours within one week of injection[3]. Further to these results, mouse tumours were injected with either recombinant anti-CD47 nanobody, sonicated SLC nanobody expressing *E. coli* or live SLC+ and nanobody expressing bacteria. Tumour growth was slowed in the first two groups but abolished entirely with whole SLC+ bacteria. These results illustrate the importance of drug delivery and the delivery system by which it uses. The self-renewing, immunogenic nature of the SLC+ bacteria doubtless played a crucial role in potentiating the immune responses to these tumours. Evidence for this was provided when the authors infected mice with A20 cells on either flank and treated only one side.

Remission was induced in both tumours and no therapeutic bacteria were detectable in the untreated tumour, indicating that tumour shrinking was induced by an adaptive immune response[3]. This is further evidence attesting to the necessity of bacterial population fluctuation punctuated by waves of PAMP, drug, and DAMP release. Potentiation of systemic immunity is itself evidence that the bacteria were in fact driving antigen release, uptake, and presentation. The fact that this was only seen in SLC+ bacteria further consolidates the notion that if bacteria are to be efficient delivery vehicles of targeted therapies to the tumour microenvironment, they have to persist for some duration, continuously deliver their gene products, and drive anti-tumour immunity.

Conclusion

Bacterial delivery of biologics is far from fantasy. Synchronised lysis circuits have been developed successfully to limit and manage the synthesis and deposition of these therapeutics to the tumour site. Furthermore, these circuits are self-limiting in nature, allowing for targeted delivery of otherwise systemically toxic agents into the tumour microenvironment. Promising in vivo evidence suggests that this method can induce tumour regression coupled with innate and adaptive immune responses. There have been some studies to date in humans, though admittedly without SLCs[16,17]. The in vivo evidence presented is currently limited to a small number of assays in murine models and requires replication. Further cancer models should be investigated, as should other therapeutic molecules. Only then will SLCs be fit for phase I human trials. Similar to a Trojan horse, we now know bacteria may infiltrate and colonize the tumour microenvironment, and now we know how to arm them. Challenges remain in finding suitable targets to differentiate malignant cells from self, and to generate efficacious nanobodies against them. In time, we may lay siege to tumours utilising these tiny Trojan bacilli. ◄

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Declarations

The author declares no conflicts of interest.

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