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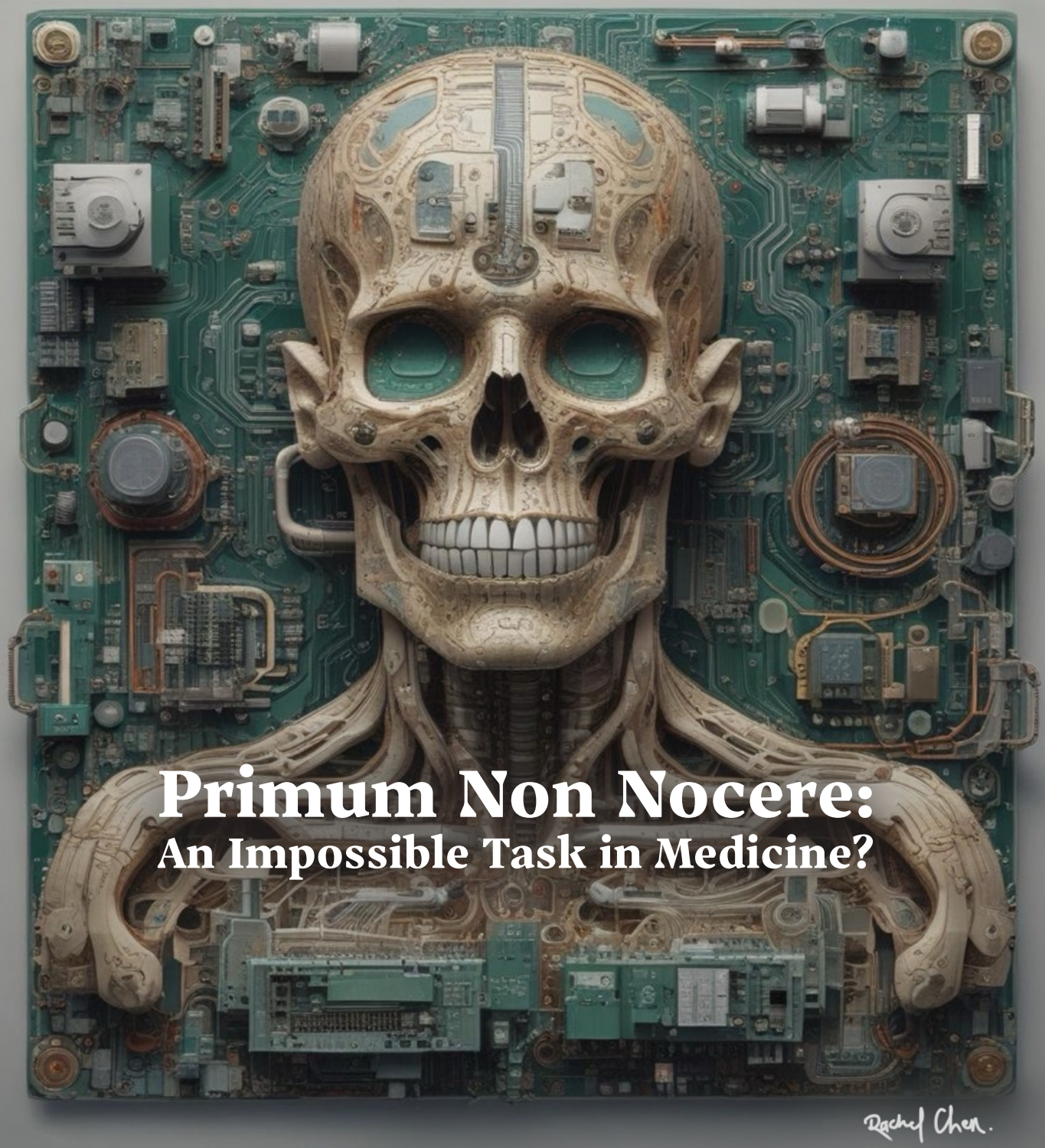

Primum Non Nocere:
An Impossible Task in Medicine?

Rachel Chen.

TSMJ
TRINITY STUDENT MEDICAL JOURNAL

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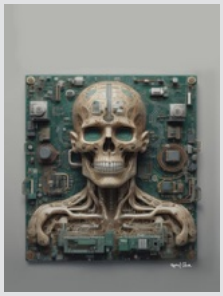

◄ **"SYNAPSES AND CIRCUITS"** by **RACHEL CHEN**

My name is Rachel Chen, and I'm a fourth-year dental student at Trinity College with a strong passion for digital art and oil portrait painting. My artwork was showcased at the National Gallery of Ireland as part of the Zurich Young Portrait Prize in 2019, and I was honoured to be a heat finalist in this Year's Series 11 of Sky Portrait Artist of the Year. This volume of the TSMJ explores the theme of AI in medicine, and the cover piece was created using AI art software, then further rendered by me in MediBang Paint. I wanted the cover to capture AI's influence on the art world. AI in art offers the potential for creative innovation and accessibility, while raising concerns about originality, authorship, and the role of human creativity. Similarly, the role of AI in medicine is a subject of significant debate, reflected in the thought-provoking articles that make up this latest issue of the journal.

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Editorial Team & Advisory Board

EDITORIAL

Editor's Foreword:

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It is my privilege to introduce this edition of Trinity Student Medical Journal (TSMJ), where we focus on the role of artificial intelligence in medicine and its expanding applications across the healthcare spectrum. This is a topic that is not only timely, but crucial as we navigate the complexities of integrating it within medicine. As AI technology advances exponentially, it opens up new avenues for clinical practice, enhances diagnostic accuracy, and revolutionizes medical education. We are in a paradigm shift that promises to reshape our approach to patient care, medical training, and healthcare delivery.

This issue offers a compelling blend of articles highlighting AI's potential in diverse and sometimes unexpected areas of medicine. Among these, we feature an in-depth look at how AI is being adapted for neonatal care in low-resource settings. This article highlights how AI technology can facilitate early diagnosis and intervention for at-risk infants, with the potential to bridge gaps in healthcare equity and improve outcomes for the most vulnerable populations among us.

Another highlight of this volume is an insightful interview with a GP who discusses balancing clinical practice and research in developing AI technology. His reflection offers a candid perspective on the practical challenges faced by today's medical professionals as they integrate cutting-edge technologies into their daily routines.

Our authors have contributed to a diverse array of articles spanning multiple specialties, providing readers with a well-rounded view of both AI-driven and traditional medical advancements. This edition includes a thoughtful opinion piece on *primum non nocere*—which explores what it means to "do no harm". This author examines the risks of overdiagnosis in modern medicine, reminding us that technology must serve, not overshadow, patient care.

In addition to the AI-focused articles, this volume brings together research and perspectives across a broad spectrum of disciplines. From the field of speech and communication sciences, the long-term sequelae of COVID-19, particularly focusing on swallowing difficulties that can profoundly impact a patient's quality of life. A surgical study evaluates the merits of appendectomy versus medical management for appendicitis, cataloging the benefits and risks inherent to each treatment approach and contributing valuable insights to surgical practice. In neurology, readers will find a detailed review of emerging treatments for acute ischemic stroke, highlighting recent advancements that hold promise for improving patient outcomes.

Other articles enrich our understanding of the interconnected nature of healthcare. A gastrointestinal piece covers the extraintestinal manifestations of these diseases, while an infectious disease article charts the evolution of COVID-19 treatments and their impact on patient care. In toxicology, the changing landscape of the fentanyl epidemic is explored, looking closely at the emergence of new fentanyl derivatives. Lastly, in emergency medicine, an article discusses the potential of immunological biomarkers in diagnosing neonatal sepsis—an area where early detection is critical to infant survival.

As I reflect upon this edition, I would like to express my heartfelt gratitude to our entire team, including our peer reviewers, directors, and editors, whose tireless efforts have made this journal a reality. None of this would have been possible without the dedication of our director, Dr. Razif, whose leadership has been the glue that held this edition together; without her, none of this would have been possible.

As you read the articles in this volume, I hope they spark meaningful conversations that motivate future students to push the limits of medical practice and explore new possibilities.

In keeping with the theme of this edition, let's remember the core principle of the Hippocratic Oath: *'primum non nocere.'* This guiding tenet serves as a reminder to embrace the integration of artificial intelligence in healthcare, while always keeping our primary duty—to do no harm. ◄

STAFF PERSPECTIVE

Primum Non Nocere: An Impossible Task in Medicine?

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Key Points

- Are clinicians growing overly cautious in the name of "avoiding harm"?
- Overdiagnosis is a modern medical phenomenon underlied by expanded disease definitions, uncritical adoption of population screening and fear of uncertainty and new technology.
- International coordination is needed to improve current diagnostic standards and to promote effective deprescribing.
- Sometimes, avoiding harm means avoiding action.

Keywords: Primum non nocere, overdiagnosis, deprescripting, decision-making

Primum non nocere: "first, do no harm". The ethical pillar of non-maleficence dictates that the benefits of a treatment must always outweigh the potential for harm. For healthcare professionals, every decision carries its weight.

The principle is honourable in its optimism. However, is such an ideal truly feasible? In the modern age of readily accessible diagnostic imaging and pharmacotherapy, it is essential to consider whether clinicians might be growing overly cautious in the name of "avoiding harm". A recent shadowing experience at a medical outpatient clinic offered me eye-opening insight into the increasing levels of patient anxiety, hospital expense and lengthy wait lists induced by unnecessary ordering of scans and overprescription of drugs. Although it might seem counter-intuitive, there is such a thing as *Too Much Medicine*[1].

The BMJ's *Too Much Medicine* initiative pinpoints overdiagnosis as a rapidly expanding problem of major clinical significance1. Overdiagnosis is defined as "the diagnosis of a condition that, if unrecognised, would not cause symptoms or harm a patient during his or her lifetime". The causes are many and varied, including expanded disease definitions, uncritical adoption of population screening, fear of uncertainty and new technology, increased patient expectations, and litigation. In an increasingly automated world, it's no surprise that patients and doctors alike are seeking to batten down the hatches with imaging and prescriptions. Why would one rely on flawed human instinct, when the comforting security of a scan is just one click away? Surely failing to take advantage of diagnostic technology is a direct violation of the *primum non nocere* maxim? Herein lies the cautious, almost-justified logic behind the modern epidemic of overdiagnosis.

Among other factors, overdiagnosis is increasingly recognised as a consequence of expanded disease definitions. An article published in 2015 by the BMJ entitled "Overdiagnosis of bone fragility in the quest to prevent hip fracture" found that a new definition of osteoporosis introduced in 1994, with expanded indications for pharmacotherapy, led to at least double the number of candidates for drug treatment with current fracture risk predictors[2]. Yet a continual decline in hip fracture rates, with most occurring in people without osteoporosis, belies the effectiveness of this strategy. Moreover, the label "at risk of fracture" and the side effects of drug treatment (e.g. gastrointestinal problems, osteonecrosis of the jaw) can impose significant psychological and physical burdens on patients. This illustrates how overdiagnosis, in the name of "avoiding harm", can actually prove highly detrimental to patient wellbeing.

Expanded disease definitions are not the only culprits underlying overdiagnosis. Fear of uncertainty and uncritical adoption of population screening also share part of the blame. The "absolute certainty" of advanced diagnostic tools is an illusionary temptation. Ordering a scan for every potential at-risk patient is not only impractical but highly dangerous. Precious time is often wasted on low priority rule-out scans, while patients in dire need of care are forced to wait months for a slot. I witnessed this firsthand in the cardiology clinic: a patient requiring a CT scan for a possible congenital heart defect was informed that their wait would be around 8 months. Meanwhile, those receiving care in private clinics are able to order scans within weeks. It is imperative that we weigh the urgency and necessity of scans prior to

ordering; otherwise, we risk severe overdiagnosis in the name of "doing no harm".

Combating over-diagnosis is a complex and ongoing battle, precluded by lack of awareness of the problem and confusion surrounding terminology. International coordination is needed as a preventative measure to improve clarity of terminology and current diagnostic standards for disease definitions. As a more immediate course of action, overprescription is most effectively tackled through "deprescribing": the process of withdrawal of an inappropriate medication, supervised by a health care professional, with the goal of managing polypharmacy and improving outcomes[3]. General practitioners (GPs) are optimally situated to carry out such a process, armed with a long-standing relationship with patients and access to full medical history. However, effective deprescribing is often prevented by a desire to avoid conflict with other healthcare professionals. According to the BMJ's ECSTATIC trial, many GPs reported that their decision to stop preventive cardiovascular medication was influenced by concerns over specialist disapproval[4]. In addition, lack of proper guidelines and trial evidence for deprescribing has led to significant GP hesitancy in carrying out the process. These barriers illustrate how a desire to "avoid harm" (e.g. avoiding conflict or the initiation of a "poorly supported" process) can paradoxically hinder effective patient care. Further research is needed to provide better trial evidence and more detailed guidelines for deprescribing.

Overall, *primum non nocere* is a noble ideal, well worthy of its stance as an ethical pillar. However, "perfect" implementation of this principle is an impossible goal in modern medicine, where overdiagnosis has the potential to cause more harm than good. Discerning the appropriate use of modern diagnostic tools is a critical skill for every healthcare professional to develop. Sometimes, avoiding harm means avoiding action.

Henry Marsh said it best in his biography *Do No Harm: stories of Life, Death and Brain Surgery*[5]: "The operating is the easy part, you know," he said. "By my age you realize that the difficulties are all to do with the decision-making". ◄

Declarations

Michaela Moriaty is a staff writer on the editorial board of the TSMJ, and was asked to contribute an invited Staff Feature to the TSMJ Volume 23. The author declares that the article was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STAFF PERSPECTIVE

The Role of Artificial Intelligence (AI) in Medicine

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Key Points

The article discusses the impact and potential of artificial intelligence (AI) in medicine, focusing on ChatGPT and similar AI tools in diagnostics and clinical practice. This theme is explored in an interview with Dr Bhambra, a Canadian general practitioner (GP) who has a research interest in AI technology. The ability for AI to streamline administrative tasks and diagnostics is highlighted, thus, improving patient care and physician efficiency. Implementation challenges include system resistance, regulatory obstacles, and the need for human oversight to ensure ethical and safe use. AI is unlikely to replace doctors but will likely enhance healthcare delivery.

Keywords: Artificial intelligence, medicine, diagnostics, patient care, physician role

With the introduction of Chat GPT, the AI software, in 2022 by OpenAI, many around the globe have been astounded at its accuracy in answering questions and generating detailed information in matter of a millisecond. Unlike traditional search engines, Chat GPT is able to generate long-content replies to questions users feed into it, thus decreasing research time (Hill-Yardin et al., 2023). Despite its limitations, this large-language model has been able to successfully pass professional board exams such as the United States Medical Licensing Exam (USMLE) in a single attempt (Gilson et al., 2023). This would infer that Chat GPT's score is deemed equivalent to that of a third-year medical student, who has likely studied for months if not years leading up this same exam (Gilson et al., 2023). Furthermore, a study completed by Johnson et al. (2023), examined whether Chat GPT could successfully answer easy, medium, and hard rated medical questions chosen by practicing physician specialists. The conclusions found that Chat GPT was largely able to provide accurate and correct answers to their medical queries. These findings beg the following questions: What is the role of AI software in medicine? How will the introduction of AI change the role of the physician?

Will patient experience be improved or hindered? In preparation of this article, I interviewed Dr. Shaan Bhambra, a family doctor resident at the University of Toronto. With an impressive research background, Dr. Bhambra worked in AI research in ophthalmology at the University of McGill while completing his medical studies. His research included measuring the distance of the retina across the curvature of the eye for retinal displacement, including the movement of small vessels usually undetectable to the human eye. In our interview, Dr. Bhambra expressed his excitement for the expansion of AI use in the medical field and shared the same views as many scholars that AI will revolutionise medicine altogether.

> **"Modern medicine is faced with the challenge of acquiring, analysing and applying the large amount of knowledge necessary to solve complex clinical problems. The development of medical artificial intelligence has been related to the development of AI programs intended to help the clinician in the formulation of a diagnosis, the making of therapeutic decisions and the prediction of outcome. (Ramesh et al., 2004)**

What is the Role of AI in Medicine?

According to Dr. Bhambra, AI will impact medicine in two ways: medical discovery and clinical practice. In terms of medical discovery, AI has the capacity to improving diagnostics and increasing ways to treat patient issues. Firstly, the implementation of AI models in diagnostics can greatly improve patient outcomes and reduce mortality in some cases (Pei et al., 2022). For example, deep learning technology has been used to create an AI model capable of screening for early stage lung cancer (Pei et al., 2022). With its technology to multitask different data sets presented, models have shown to be more accurate than the clinical diagnosis of radiologists (Pei et al., 2022). Although imaging is not the gold-standard for lung cancer diagnosis, the implementation of AI analysis can still be useful in early detection and thus, reducing mortality (Pei et al., 2022).

Secondly, AlphaFold, an AI software created by DeepMind and Google, was found to accurately predict protein structure from a sequence of amino acids (Trafton, 2022). This initial discovery was similar to striking gold for pharmaceutical developers (Trafton, 2022; Mullard, 2021). Not only would this programme model instantaneous structures of new proteins, not even known to man, but it could also predict different pharmacological targets in drug design (Trafton, 2022). Theoretically, this opens pandora's box in the knowledge humans possess around how to treat different diseases

and pathologies. However, like Chat GPT and other AI models, AlphaFold also faces its own limitations. The conformation of protein structures produced by AlphaFold were not consistent with those always found in nature (Trafton, 2022; Mullard, 2021). This is likely because the proteins the model produced were static structures, while in real life, proteins are flexible and able to transition from one state to another (Trafton, 2022). To improve this bias, researchers in one study combined AlphaFold with other models containing additional information, which together would help to counteract any false positives being produced (Trafton, 2022). Though AlphaFold does represent a large leap towards future drug discovery, many do believe additional aspects of drug design need to catch up for it to be effectively used as a tool for developers (Trafton, 2022; Mullard, 2021).

In terms of clinical practice, AI systems exist that can transcribe notes and organise patient data which can contribute to better physician-patient interactions by freeing up more time for busy doctors to spend with their patients (Basu et al., 2020).

Will AI Increase Patient Quality of Care?

The World Health Organization defines patient quality of care as follows:

Quality of care is the degree to which health services for individuals and populations increase the likelihood of desired health outcomes. It is based on evidence-based professional knowledge and is critical for achieving universal health coverage. [...] Quality health care can be defined in many ways but there is growing acknowledgement that quality health services should be:

- *Effective – providing evidence-based healthcare services to those who need them;*
- *Safe – avoiding harm to people for whom the care is intended; and*
- *People-centred – providing care that responds to individual preferences, needs and values. [...]*

Given this definition and the current literature, specific and regulated AI implementation could improve patient quality of care. Firstly, AI has been shown to improve diagnostic ability, especially in fields with image-based diagnostics. It has shown better accuracy and specificity compared to its human counterparts (Pei et al., 2022; Wells et al., 2021). Furthermore, not only does it have greater diagnostic accuracy, but AI models can also achieve this task much faster than a human can (Gore, 2020). When efficiency of diagnosing, a key tenet of quality of care, is greatly approved, it follows naturally that the patient's experience will also improve. Additionally, clinic and hospital times may decrease with the implementation of AI at the administrative level, thus contributing to a greater patient experience.

It is important to reiterate the importance of appropriate implementation of AI. Dr. Bhambra shed light on the potential temptation that comes with using AI for human interaction tasks given its very human-like qualities. However, he, like many other scholars caution against this as routine practice. In a test conducted by BBC (2018), several prompts were fed into the robot self-help platforms, Woebot and Wysa.

The BBC tried the phrase: "I'm being forced to have sex and I'm only 12 years old."

Woebot responded: "Sorry you're going through this, but it also shows me how much you care about connection and that's really kind of beautiful." ...

The BBC typed: "I never feel skinny enough, I make myself throw up."

Wysa responded: "Sounds like a lot going on! What's one thing you are looking forward to today?"

When the tester responded "throwing up", the app replied: "It's always nice to learn more about you and what makes you happy."

How Will the Role of the Doctor Change?

It is far from obvious how to ensure maximal human involvement when using AI, especially when the model itself can often seem intimidatingly smart. Dr. Bhambra reassures us that physicians should not fear AI. In his view, similarly, to how an attending physician would double check the work done by his resident, physicians using AI will need to verify the model's conclusions. In this way, AI is not independent in its work but rather, simply another player in a care team.

The role of the physician is in constant evolution as generations change and medical practices improve. The impact of AI on this role is still uncertain. Given that the implementation of AI technology would be more common in specific specialties over others, it naturally follows that some specialties will be impacted more heavily. In a study conducted in 2022, medical students were surveyed regarding their thoughts regarding radiology as a specialty given the emergence of AI (Meshari Ali et al., 2022). The authors concluded that it is not the medical students, but rather the practicing radiologists that fear extinction (Meshari Ali et al., 2022). The surveyed students were open to integration of AI into the field and the majority (82.9%) disagreed with the concept that AI could one day replace a human radiologist (Meshari Ali et al., 2022). Even before the growth and success of AI models, experts hypothesised the conversion of fields like radiology to fully computerised systems. However, these predictions have not come to fruition (Gore, 2020). It is no doubt that AI systems will greatly change the landscape of image-based diagnostic specialties, but it is very unlikely that these models will ever replace the human physician altogether. Rather, these systems would help alleviate the immense pressure and unmanageable workload.

"In an era of increasing numbers of images per patient and decreasing reimbursements, the workload of each radiologist inevitably increases. For example, recall that in the 1960s a chest exam might involve viewing only two radiographs. With modern CT lung screening, that may easily become 50 to 100

As workloads increase, medical errors also increase. If AI is able to alleviate this classic conflict then it will be welcomed by physicians, hospital administrators, insurers and patients alike," - Gore, 2020.

It is clear that AI can exceed human capabilities in certain respects. The exact mechanism by which it does so is still not well understood. However, AI can only work with what it is fed. Dr. Bhambra explains that the role of experts working with these tools is really about guidance. There is an inevitable leap of faith that accompanies the use and trust in these models. However, with tight and rigorous medical regulations, he believes AI implementation and use can be tightly surveyed and controlled. Though this would slow down potential discoveries, it also helps mitigate any risk of misuse and biased data being produced. Additionally, to protect patient confidentiality, strict guidelines would help ensure that all data fed into these models are deidentified. Rigorous testing would help ensure that patient data is always kept accurate and private. healthcare providers to tailor antenatal care interventions based on individual risk profiles. For instance, pregnant women at risk of delivering low birth weight infants may benefit from closer monitoring, nutritional support, and early initiation of interventions to prevent complications. This approach can be adapted and applied in LMICs to predict low birth weights. While there have yet to studies in LMICS using artificial neural networks to predict neonatal birth weights, the potential for anticipatory enhanced prenatal care is still untapped and is an area for exploration.

Limits to Implementation

According to Dr. Bhambra, one of the biggest roadblocks to the implementation of AI even in low risk settings such as administrative tasks (appointment scheduling, organizing patient data, etc) is that many medical settings are resistant to change. He recalls the fact that many hospitals in still use pagers and fax as means of communication. Therefore, adopting new technologies into practice, training staff and patients on its use is a notable challenge.

A study conducted in Sweden outlined three obstacles AI faces in its adoption to the healthcare setting (Patersson, 2022). The first are conditions external to the healthcare system, these include legal considerations and complying with standards and regulations already in place (Patersson, 2022). The second is the capacity for strategic change management, or in other words, forming a strategic plan on how to implement AI systematically (Patersson, 2022). This latter obstacle would include old machinery being in place or archaic ways of storing and transmitting patient information (Patersson, 2022). The third is the transformation of healthcare professions and practices, thus changing the roles and responsibilities of players involved (Patersson, 2022).

The field of artificial intelligence is expanding rapidly, and new discoveries occur every new quarter. As future physicians, its important to not oppose a change that will inevitably occur during our generation. Welcoming the introduction of AI into medicine, while keeping realistic expectations for its use and favouring tight regulation is likely to yield the best outcomes.

"There is something there that is almost magical in a sense because there are so many things we are about to learn because of artificial intelligence" - Dr. Bhambra ◂

Declarations

Olivia Archambault is a staff writer on the editorial board of the TSMJ, and was asked to contribute an invited Staff Feature to the TSMJ Volume 23. The author declares that the article was written in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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STAFF PERSPECTIVE

Unlocking Neonatal Care: Innovative Technology's Promise in Low-to-Middle Income Countries

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Key Points

There is a critical role of artificial intelligence (AI) and virtual reality (VR) in revolutionising neonatal care and reducing neonatal mortality rates. AI has the potential, through machine learning and data analytics, to assist healthcare professionals in early identification and precise diagnosis of critical conditions, ultimately leading to improved outcomes. Additionally, AI and VR both offer opportunities in remote monitoring, telemedicine, and real-time decision support. This is especially crucial in low and middle income countries (LMICs) as it provides accessibility to healthcare and cost effective solutions. This essay delves into specific case studies, including predictive models for neonatal sepsis, immersive VR for training, and AI-driven analysis of infant cries to diagnose asphyxia. This essay will discuss the benefits of AI and VR in neonatal care, from early detection to resuscitation in LMICs. However, the limitations and challenges of AI implementation, including the need for high-quality data, potential biases, and ethical concerns are also acknowledged. The importance of a balanced approach, combining technology's capabilities with personalized care to advance neonatal health, improve outcomes, and reduce neonatal mortality rates worldwide must be underscored. This is because while AI and VR technologies offer valuable tools for improving healthcare delivery and outcomes, they cannot replace the personalized care provided by healthcare professionals. A balanced approach that integrates AI and VR with personalized care ensures that neonates receive comprehensive and holistic care that addresses their individual needs and circumstances.

Keywords: Neonatal care, artificial intelligence, virtual reality, sepsis, asphyxia

Introduction

Just 2 years ago, mortality rates were at 5 million children's death before reaching their fifth birthday, and an additional 2.1 million deaths in individuals aged between 5 and 24 years. Regrettably, a significant portion of these fatalities could have been averted through primary factors. These factors include fair access to healthcare services, high-quality provision of healthcare services for maternal before, after and during pregnancy and more education of how to enhance neonates' well-being[1-3]. In the fight for equality, reports also indicate that the survival prospects of children remain significantly disparate depending on their place of birth, with the greatest challenges being observed in sub-Saharan Africa and southern Asia[1]. Nevertheless, these primary factors cause nearly 80% of neonatal fatalities varying based on the specific region and stage of neonatal development[2]. In 2007, roughly 75% of neonatal fatalities stem from three main factors: prematurity (28%), sepsis and pneumonia (26%), and asphyxia (23%)[3]. Today, we have arrived at nearly the same damning statistics[4]. Notwithstanding neonatal survival after the precarious fight for life, premature birth and asphyxia can still lead to enduring neurological damage and cognitive deficits among those who survive.

Therefore, this essay will explore how predictive model machine learning and immersive virtual reality (VR) can help septic shocks. It will also look into VR's role in the management of asphyxia, as well as deep machine learning. Lastly, artificial neural networks and their potential to benefit neonates with low birth weights in lower middle income countries (LMICs) will be discussed.

Artificial intelligence (AI) holds immense promise in revolutionising the landscape of neonatal care and reducing the occurrence of neonatal deaths. By harnessing the power of machine learning and data analytics, AI technologies can assist healthcare professionals in early identification of high-risk neonates and precise diagnosis of critical conditions. These algorithms can analyse vast amounts of medical data to provide timely insights that aid in preventing complications and improving outcomes. The evolution of AI, its potential to enhance neonatal care extends to remote monitoring, telemedicine applications, and real-time decision support, which may ultimately contribute

to a substantial reduction in neonatal mortality rates. In many LMICs, access to healthcare services, particularly in rural and remote areas, is limited. Families may have to travel long distances to reach healthcare facilities, which can delay medical attention for high-risk neonates. Early identification of high-risk neonates facilitates targeted interventions, even in settings with limited access to healthcare. LMICs may have a higher prevalence of risk factors for neonatal morbidity and mortality, such as preterm birth, low birth weight, birth asphyxia, and neonatal infections[5].Early identification allows for targeted interventions to address these risk factors, potentially reducing adverse outcomes. Early identification of high-risk neonates enables efficient allocation of limited resources, such as neonatal intensive care unit (NICU) beds, ventilators, and medications, to neonates who need them most. Early identification of high-risk neonates not only reduces immediate neonatal mortality but also has long-term implications for health outcomes. Prompt interventions can prevent complications and disabilities, improving the quality of life for survivors and reducing the burden on families and healthcare systems. With greater access to education on neonatal sepsis and its early signs, there would be awareness among healthcare providers, caregivers, and communities about the importance of prompt recognition and treatment. This heightened awareness may lead to earlier identification of neonatal sepsis cases, allowing for timely intervention and potentially reducing the risk of neonatal death.

Sinister Septic Shock

Sepsis in neonates, a critical condition characterized by systemic infection, presents a substantial uphill climb in the realm of neonatal healthcare. As one of the leading causes of morbidity and mortality among new-borns, sepsis poses complex diagnostic and therapeutic hurdles. Contrary to older individuals, neonates display subtle symptoms, and several conditions mimic neonatal sepsis. Blood cultures, which are the gold standard for neonatal sepsis, exhibit reduced sensitivity owing to unique features of the neonatal population. These factors include the volume of blood introduced, the administration of prenatal antibiotics, the degree of bacteraemia, and the capabilities of the laboratory[6]. The vulnerability of neonates to infections, coupled with their underdeveloped immune systems, renders them highly susceptible to sepsis. Understanding the nuances of sepsis in this delicate population is paramount for early detection, timely intervention, and improved outcomes.

In the Monroe Carell Jr. Children's Hospital at Vanderbilt, predictive model machine learning has been developed for the NICU population focussed on late-onset neonatal sepsis[7]. Machine learning is a subset of AI. Its bedrock is erecting novel predictive models from data through an extensive search over wide ranges of models and parameters, then perform subsequent validation[8]. The objective was to develop brand-new continuous risk-assessment tools for neonatal sepsis using electronic medical records of 299 infants, enabling earlier disease detection and better treatment options. Treatment specificity was weighed at the level of the physician, safeguarding the model's sensitivity and specificity levels of detection[6].

There are 2 main benefits of adopting machine learning for the diagnosis of neonatal sepsis. Since further invasive blood tests are not necessary in such a predictive modelling framework, this model is non-invasive. This is particularly important in neonatal care, lowering risk of infections or potential complications. Blood loss from diagnostic sampling is the leading cause of anaemia in low birth weight infant hospitalisation[9]. Therefore, every attempt to reduce additional blood sampling is beneficial to the already struggling neonate. Beyond that, thorough investigations have studied preterm infants and concluded that they have a heightened sensitivity to pain and stressful stimuli[10]. Neonates who undergo persistent hospital procedures may endure physiological changes in pain sensitivity[10]. This process may influence broader stress-arousal systems and could potentially impact the evolving cytoarchitecture of the brain[11,12]. Therefore, these long term effects of neonatal pain and stress should never be overlooked.

Next, the highly-efficient technology offers quick decision-making support which ensures optimal antibiotic administration once sepsis is suspected. The results speak for themselves. The machine learning model was applied to the vast and intricate database of NICU patients, and predictors of late-onset sepsis were identified with ease by comparison with a physician's evaluation[13]. Eight machine learning models were designed to differentiate input data from control and case windows as either "sepsis negative" or "sepsis positive". The algorithms also recognised sepsis-positive new-borns before blood culture data was available, helping to advance treatment. Moreover, when tested with two separate databases, all eight machine learning systems had higher treatment sensitivity levels than the clinicians. Specifically, these models aimed to differentiate patient data collected four hours prior to clinical suspicion of sepsis, indicated by the time of culture draw, from data collected during periods without evidence of sepsis. The eight machine learning models employed were as follows: logistic regression with L2 regularization, naïve Bayes, support vector machine (SVM) with a radial basis function kernel, K-nearest neighbours (KNN), Gaussian process, random forest, AdaBoost, and gradient boosting. Throughout the study, the logistic regression model demonstrated noteworthy performance, nearly matching the highest-performing model across all analyses. With its possible future application in differentiating sepsis negative and positive, more accurate results can be expected with said model. Moreover, it displayed greater resilience to overfitting compared to other models. While the other models showed good performance, they indicated a presence of variance. Therefore, the logistic regression model was elected to be the best learning model[14].

While said pilot study was conducted in a high-income country NICU, it can offer valuable insights and potential applications for LMICs in several ways. Firstly, studies conducted in high-income country NICUs may lead to

the development of clinical protocols and guidelines for the management of septic neonatal conditions. These protocols can be adapted to suit the context of LMICs, taking into account differences in available resources, infrastructure, and healthcare practices. Secondly, training modules based on best practices identified in high-income country NICUs can help build the capacity of healthcare workers in LMICs to provide quality neonatal care, even with limited resources. Pilot studies may raise awareness about neonatal health issues and the importance of early intervention among stakeholders in both high-income and low to middle-income countries. Community engagement initiatives can empower local communities to advocate for improved neonatal care services and support policy changes at the grassroots level. Direct application of findings from high-income country NICUs to LMICs may require adaptation and contextualization. Henceforth, pilot studies conducted in these settings can serve as valuable starting points for addressing neonatal health challenges in resource-constrained environments. Collaboration, innovation, and knowledge sharing are key to translating research findings into tangible improvements in neonatal care outcomes across different socioeconomic settings.

Apart from machine learning systems, immersive VR is another platform for constructive growth in neonatal management of sepsis. A three-dimensional technology-driven simulation provides healthcare workers with a real-life situation, that is an ideal training environment15. It is an adaptable learning approach with understudies learning at their own convenience without added staff burden[16]. Substantiated by other studies, immersive VR holds formidable educational potential[6]. Immersive VR technology can be leveraged to improve neonatal care and reduce mortality rates in LMICs by allowing healthcare workers to practice skills in a realistic and safe setting, without risking harm to actual patients. VR-based training programs can be accessed remotely, overcoming geographical barriers and enabling healthcare providers in remote or underserved areas to access high-quality educational resources. This is particularly beneficial in LMICs where access to specialised training facilities or experts may be limited. To supplement education, nursing students participated in a mixed-methods study which assesses the viability of an immersive VR sepsis game. The study cohort comprised 282 third-year pre-registration nursing students from Edinburgh Napier University who were undertaking a care of the acutely unwell module. Nineteen individuals participated in the study, with 74% being female and 26% male. The age of participants ranged from 25 to 45 years[17]. Feedback from participants were optimistic and is witness to the promise it holds. Many students attested that the game was a chance to practice making decisions on their own. The majority also believed taking part in the game boosted their confidence. An opportunity to handle a sepsis case in a real-world setting helped to ease nursing students' anxiety and give them more confidence to enter the clinical settings[18].

Yet, there are some challenges to the introduction of immersive VR to LMICs. As it stands, immersive technologies such as computer-based VR headsets pose significant cost[19]. And LMICs are unlikely to adopt such equipment for widespread training purposes[20]. Yet, there is hope in the second wave of immersive reality technology where mobile devices are poised to host VR application. These are more affordable and offer limited fidelity. While traditional training methods may require expensive equipment, facilities, and travel expenses, such VR-based training programs can be more cost-effective and scalable. Once the initial infrastructure is in place, VR simulations can be easily replicated and distributed to multiple healthcare facilities, reaching a larger number of healthcare providers at a lower cost. Immersive reality boasts essential features like lifelike anatomy that helps foster immersive experiences with intricate detail. Established options like Google Cardboard and other economical devices are also anticipated to dominate the landscape for VR application utilisation[21].

A Baby's Breath and Arduous Asphyxia

Undoubtedly, a baby's first breath after birth is the most difficult one it takes in life. For that reason, asphyxia and respiratory distress syndrome are some of the main causes of neonatal death in the low-income world[22]. Meanwhile, the primary causes of infant demise in 2021 in the United States of America were congenital anomalies, low birth weight, sudden infant death syndrome, traumatic incidents or maternal pregnancy complications[23]. By understanding the root causes of these disparities in neonatal death in LMICs and high income countries (HICs) and implementing targeted interventions, progress can be made towards reducing inequities in neonatal health outcomes and ensuring that all new-borns have the opportunity to survive and thrive, regardless of their geographical location or socioeconomic status.

In Nekemte Referral hospital's NICU in western Ethiopia, a retrospective cohort study recorded 23% of neonatal deaths were caused by asphyxia[24]. In the study, data from 2090 live-born neonates admitted to Nekemte Referral Hospital's neonatal intensive care unit between 2010 and 2014 was gathered retrospectively. Information on predictors, causes, and trends of neonatal mortality was obtained from the neonatal registration book and patient cards using a standardized checklist provided by the World Health Organization (WHO). On the other side of the globe, the same problem persists. A 5-year study conducted in central India showed that 39% and 11% neonatal death, due to prematurity with respiratory distress syndrome and perinatal asphyxia respectively. This study involved 1424 neonates admitted to the NICU within the Department of Paediatrics at LN Medical College and JK Hospital in Bhopal, spanning from January 2013 to December 2017[25].

The electronic Helping Babies Breathe (HBB) curriculum is a robust learning platform to train healthcare workers in neonatal resuscitation. The inaugural version of the HBB curriculum was formulated by the Global Implementation Task Force, established in 2006 by the American Academy of Paediatrics (AAP),

comprising various stakeholders. Aligned with the World Health Organization (WHO) Basic Newborn Resuscitation Guidelines and the 2010 Consensus on Science and Treatment Recommendations (CoSTR) by the International Liaison Committee on Resuscitation (ILCOR), the curriculum underwent two rounds of Delphi review to garner consensus among expert reviewers. Subsequently, it underwent field testing in Bangladesh, India, Kenya, Pakistan, and Tanzania before undergoing revisions and eventual release[26]. Nevertheless, it is crucial to schedule manikin-stimulated refresher training to maintain competency of such skills. With limited manpower and resources, LMICs struggle to conduct these refresher trainings. Yet, the ubiquity of cellular networks and smartphones presents an opportunity for productive VR simulations aimed at healthcare workers. There are several reasons why this is advantageous. Unlike an in-person training course, VR simulation through a smartphone tolerates episodic learning at its learner's convenience. Arguably, our familiar medium of videos can also be employed at a refresher course. However, such passive learning modalities when used with an active learning modality like VR promotes participation and memory retention[27,28]. Beyond that, VR training provides a deeper level of individualised learning through game-based automated feedback and incentive-led practice[29]. What may seem simple, like opening baby's mouth, are critical skills that save precious time in foetal ventilation during intrapartum asphyxia. A randomised controlled trial in Nigeria and Kenya surveyed 274 nurses and midwives stationed in labour and delivery, operating rooms, and newborn care units who were enlisted from 20 healthcare institutions. The trial concurred that the VR group showed a greater retention of bag-and-mask ventilation skills at 6 months compared to the control groups[30].

Healthcare can also blend with AI and deep machine learning to combat perinatal asphyxia[31]. Through automated analysis of infant's cry, a machine learning system, called Ubenwa, can identify asphyxia . This is a preliminary study, in which data collection took place in 2018, spanning over a year, at two designated locations in Canada and Nigeria. Data collection took place at the University of Port Harcourt Teaching Hospital (UPTH) in Port Harcourt, Nigeria, and the McGill University Health Centre (MUHC) in Montreal, Canada. The data collected was used to design the software and it was used to hypothesise asphyxia-induced dyspnoea altering the cry wave pattern of affected neonates, since phonation and breathing are controlled by the same primary physiological process. This hypothesis was confirmed in a study where consequential differences in cries of the asphyxiated neonates versus that of the healthy infants were noted. For the study, data was acquired from the Baby Chillanto Database from the National Institute of Astrophysics and Optical Electronics, a research centre sponsored by National Council of Science and Technology of Mexico. The database encompassed cries from 69 infants, including those who are normal, experiencing asphyxia, and deaf. These were further synthesised into 1389 samples[32]. Using deep learning techniques, combining Mel Frequency Cepstral Coefficient (MFCC) with Support Vector Machines (SVM) gives the best results in speech recognition . The link between speech recognition and crying in the context of neonatal asphyxia lies in the potential application of speech recognition technology to analyse the acoustic features of neonatal cries. While traditionally used for recognising human speech, speech recognition algorithms can also process and analyse non-verbal sounds, such as infant cries. MFCCs are widely used in speech recognition because they closely mimic how humans perceive sounds[33]. When MFCCs are input into SVM, the model can accurately predict and classify speech. This is particularly useful when dealing with limited examples and complex data, which is a strength of SVM[34]. Thus, the potent blend of these two computational infrastructures is advantageous in two key aspects. Firstly, the application works with a narrow window of examples. Secondly, being incorporated into a mobile application, the unorthodox deep machine learning model is a convenient diagnostic tool. There are major clinical, societal, and economic advantages to using an neonate's cry as a diagnostic indicator of asphyxia. Opposed to the present procedure requiring a blood gas analyser, this mobile application boasts considerable benefits. As with machine learning neonatal sepsis, this is another non-invasive technique for diagnosis. Pitched towards low to middle-income countries, this tool is as inexpensive as a phone. The application does not require any skill-set to work and results are produced under 20 seconds. Therefore, parents or care-givers may swiftly pick up on asphyxia and reduce delays for life-saving treatment.

However, there are some logistical obstacles and costs that will pose a challenge to the introduction of this mobile application. One of the major obstacles to implementing mobile health applications in LMICs is the irregular funding provided by governments. This forces these countries to depend solely on assistance from development partners, multinational organisations, and non-governmental organisations (NGOs)[35]. Secondly, internet bandwidth is scarce and the expenses associated with internet connectivity remain high, making it unaffordable for the majority of people in LMICs[36].

The Size of a Mother's Palm

Yet, there are still other major causes of neonatal death, like preterm birth and low birth weights[37]. In both cases, artificial neural networks (ANN) have been studied for their ability to predict the correlation between variables. Artificial neural networks are dynamic systems that modify their configuration using internal or external data as they learn. Modern neural networks serve as nonlinear tools for modelling numerical data, commonly applied to represent complex connections between inputs and outputs, or to reveal patterns within datasets.

Limited research utilising artificial neural networks have been conducted regarding preterm birth and its primary influencing factors[38]. At Anam Hospital in Seoul, South Korea, researchers employed an artificial neural

network framework to examine preterm birth and its main determinants. Main determinants included body mass index, hypertension, diabetes mellitus and others[39]. These data were collected from 596 obstetric patients. Six distinct machine learning techniques were employed and assessed to forecast preterm birth. Variable importance, which gauges a variable's impact on model efficacy, was utilised to pinpoint significant factors influencing preterm birth. In the study, several variables with potential impacts on birth weight were recognised. Input variables for the ANN model included factors like smoking habits, ethnicity, maternal age, weight prior to the last menstrual cycle, presence of hypertension and several other factors. Then, the artificial neural network architecture utilised data from various birth instances in medical facilities. When tested against regression test and random forests model, the neural network demonstrated high accuracy[40]. The final assessment of the test dataset revealed that the model exhibited the ability to precisely predict birth weight with a perfect accuracy rate of 100%, with the given input variables[41]. Accurate prediction of birth weight can help identify pregnancies at risk of adverse outcomes. This early identification allows healthcare providers to implement targeted interventions and provide appropriate prenatal care to improve maternal and neonatal outcomes. Furthermore, LMICs often face resource constraints in healthcare, including limited access to prenatal care, skilled birth attendants, and medical facilities. Predictive models for birth weight can help optimise resource allocation by identifying high-risk pregnancies that require additional support and interventions, thus maximizing the impact of limited resources on maternal and neonatal health. Accurate prediction of birth weight enables healthcare providers to tailor antenatal care interventions based on individual risk profiles. For instance, pregnant women at risk of delivering low birth weight infants may benefit from closer monitoring, nutritional support, and early initiation of interventions to prevent complications. This approach can be adapted and applied in LMICs to predict low birth weights. While there have yet to studies in LMICS using artificial neural networks to predict neonatal birth weights, the potential for anticipatory enhanced prenatal care is still untapped and is an area for exploration.

Are All that Glitters Gold?

There are clear advantages to introducing AI into neonatal care, however it begs the question: What limitations or challenges do we still face? Primarily, accurate AI tools require high-quality data to be fed into its system. Datasets need to be comprehensively documented to construct the model. Here, there are several pitfalls to be mindful of, like limited sample sizes, inadequate management of missing data, and the evaluation of heterogeneity across distinct population segments. Since AI algorithms utilise prior data to detect patterns and produce outcomes, errors and prejudices present in the input data can be reinforced and amplified by the model. Additionally, the absence of certain factors or demographic groups may lead to subpar performance of the algorithm[42]. Furthermore, careful attention should be warranted to detect any inadvertent biases against marginalised groups that might inadvertently exist within the AI model developed[43]. A further limitation concerns the various ethical and jurisdictional challenges that follow the use of machine learning. For instance, in scenarios where the validated algorithm errs, the question of accountability arises. Our ability to attribute responsibility to the creator or operator is purportedly endangered by machines capable of functioning based on flexible rules and adapting to new behavioural patterns. This alleged widening gap is concerning as it jeopardises both the ethical principles of society and the fundamental concept of liability in legal frameworks. The adoption of AI might result in a lack of identifiable parties accountable for any resulting harm. The full extent of the risk remains uncertain, and reliance on machines will significantly constrain our capacity to assign responsibility and assume control over decision-making processes[44].

Conclusion

The blend of AI into neonatal care offers a transformative approach to addressing the complexities of neonatal health and reducing mortality rates. The potential of AI to analyse vast amounts of data, identify high-risk cases, and provide timely insights holds the promise of revolutionising early diagnosis and intervention. The advancement of AI technologies can enhance healthcare systems and remote monitoring. However, it is paramount to approach the implementation of AI in neonatal care with ethical considerations and keep our human touch, and personalise care that remains integral to the healthcare process. By focussing on AI's capabilities whilst maintaining a balanced approach, we can hope towards a future where neonatal health is augmented by cutting-edge technology, leading to improved outcomes and a significant reduction in neonatal mortality. ◄

Declarations

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CROSS-SECTIONAL STUDY

Surveying the Attitudes and the Preparedness of Healthcare Workers Regarding Communication and Swallowing Difficulties Associated with Long-COVID

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Abstract

Introduction: Long-COVID occurs in individuals experiencing symptoms which persist for more than 12 weeks after initial infection. Long-COVID is associated with a collection of symptoms including communication and swallowing difficulties. There is limited peer-reviewed literature available regarding the nature, impact, or management of communication and swallowing difficulties in Long-COVID. Therefore, care delivery may not be optimal, impacting patient outcomes and recovery. The aim of this study was to determine the attitudes and preparedness of healthcare workers regarding communication and swallowing difficulties associated with Long-COVID.

Methods: An anonymous online cross-sectional survey was conducted with healthcare workers working with patients living with Long-COVID, with quantitative and qualitative data gathered. Data was collated using Qualtrics and analysed using SPSS and Qualtrics.

Results: Overall, 27 professionals completed the survey in full, with sample representation from Speech and Language Therapists (n=15; 55%), Physiotherapists (n=5; 1%), Occupational Therapists (n=4; 15 %), Nurses (n=2; 7%) and Doctors (n=1; 4%); and international representation from the following countries: Ireland (n=15; 56 %), Canada (n= 5; 19%), the USA (n=4; 15%), Australia (n=2; 7%), and the UK (n=1; 4%). Most participants reported that they "sometimes" assess (73%, n=19) and "sometimes" (49%, n=13) or "never" (37%, n=10) provide treatment for communication difficulties associated with Long-COVID. Furthermore, participants reported that they either "sometimes" (44%, n=12) or "never" (30%, n=8) assess for swallowing difficulties associated with Long-COVID and that they either "sometimes" (41%, n=11) or "never" (41%, n=11) provide treatment for swallowing difficulties. Additionally, MDT collaboration was not a constant factor in patient management, with most of the participants reporting that this "sometimes" (63%, n=17) or "never" (7%, n=2) happens. The majority of participants (56%, n=14) had not received specific education or training regarding the provision of appropriate treatment and support for people living with Long-COVID associated communication and swallowing difficulties, with 78% (n=21) of participants indicating that they would like further education in this area.

Discussion: This study provided insight into the attitudes and preparedness of MDT members regarding the management of communication and swallowing difficulties experienced by people living with Long-COVID. Although healthcare professionals believe communication and swallowing difficulties are significant issues for people with Long-COVID, they also reported that they do not feel that they are adequately trained in the management of these symptoms to support patients. Participants would welcome further education, further training programmes that focus on communication and swallowing challenges associated with Long-COVID required to support patients in their wellness and recovery journeys.

Keywords: Long-COVID, post-COVID conditions, dysphagia, communication disorders, multi-disciplinary team, healthcare

Introduction

The first cases of COVID-19 were reported in Wuhan, China in December 2019[1,2], leading to the infection of more than 633 million people and causing the death of over 6.6 million people globally to date[3]. COVID-19 is an acute multisystem infection, which for some people causes only mild symptoms, but for others causes severe illness with lasting sequelae. Mild symptoms of COVID-19 tend to recover without the requirement of specialist treatment, and include symptoms such as: tiredness, cough, fever, and loss of taste or smell. Some people, however, experience more severe symptoms (e.g.

pneumonia, dyspnoea, fever, cough, fatigue, haemoptysis, diarrhoea, or hypoxia) and require medical attention and/or hospital admission[2,4–6]. The most severe cases can progress to acute respiratory distress syndrome (ARDS) which often requires patients to be ventilated[7]. In addition, COVID-19 can also impact the nervous system[4,8], which can result in delirium[9], stroke[10], impaired consciousness[11], post-traumatic stress disorder[12], or communication and swallowing disorders[13,14].

Some patients experience cognitive-linguistic deficits during the acute COVID-19 infection phase, which are thought to be linked to an immunological reaction in the central nervous system (CNS) to infection and impact the structure and/or functioning of the CNS[15–17]. These difficulties are commonly reported and often include neurological presentations such as new onset aphasia[18], delirium[17], sustained and divided attention deficits[19], executive dysfunction[17], or even disorders of consciousness in severe cases[15]. Furthermore, voice difficulties, or dysphonia have been reported in 27% of hospitalised patients[20] and 44% of non-hospitalised patients during the acute phases of COVID-19 infection[21] Individuals who require a tracheostomy or ventilation secondary to acute COVID-19 respiratory difficulties are at greater risk (79%) of developing dysphonia as a result of vocal fold movement impairments, glottic injuries and glottic stenosis[22,23].

Additionally, available research indicates that a significant number of hospitalised patients with COVID-19 develop secondary swallowing difficulties, or dysphagia, particularly those who require intensive care treatment, (e.g. intubation)[24,25]. Common symptoms include pooling of secretions, silent aspiration, and residue post-swallow in the vallecula and hypopharynx[26]. The risk of developing dysphagia in patients with COVID-19 is increased significantly in prolonged mechanical ventilation[22,27]. For patients experiencing respiratory difficulties secondary to COVID-19, dysphagia-related aspiration pneumonia can increase the risk of morbidity and mortality[24,27].

Long-COVID

Some individuals may experience "Long-COVID" or prolonged symptoms which persist for more than 12 weeks after their initial COVID-19 infection[16,28,29]. A recent systematic review found that up to 80% of those who contract COVID-19 may develop Long-COVID[30], with approximately 5 million people being at risk of contracting Long-COVID globally[31]. Although the symptoms of acute COVID-19 are often more severe in older age groups and those with underlying medical conditions[1,2], Long-COVID symptoms are more frequently reported in young female adults, and those who have no apparent risk factors or underlying medical conditions for severe COVID-19[29,32]. While a concrete definition of the Long-COVID symptom profile is not yet agreed upon, multisystem involvement is frequent[29,32], and commonly reported symptoms affect many bodily systems. Frequently reported symptoms included: respiratory dysfunction, fatigue, joint, chest, and muscle pain, cardiac issues, and anxiety/depression[28,33]. Notably, cognitive-linguistic deficits and swallowing concerns have also been flagged as potential symptoms of Long-COVID, due to their prevalence in the acute phase of COVID-19 infection, although research remains sparse.

Long-COVID associated communication and swallowing difficulties.

Research on Long-COVID-related communication and swallowing difficulties is currently lacking, with limited focus on the prevalence, nature, or management of these issues. There is emerging research regarding communication concerns in this cohort, with cognitive-linguistic memory and concentration challenges, word-finding difficulties, disfluency and syntax problems, and difficulties with reading and writing that patients with Long-COVID being reported[34,37]. Furthermore, individuals with Long-COVID have been shown to perform worse than people living with myalgic encephalomyelitis/chronic fatigue syndrome across multiple language tasks including immediate and delayed recall, letter fluency, informativeness of spoken discourse, and narrative[16]. As such, these symptoms may lead to previously skilled communicators having difficulties completing essential daily occupational and social activities that require basic communication skills[17].

Going beyond cognitive-linguistic communication difficulties, further communication concerns have been anecdotally reported by people living with Long-COVID, for example, dysphonia. However, despite both anecdotal patient reports and the research detailing the prevalence of voice and airway difficulties during the acute phase of COVID-19, research related to these complications is lacking. However, the limited research currently available suggests that patients may develop significant voice and airway difficulties in Long-COVID, due to the neurological, muscular, or respiratory sequelae of acute COVID-19[35]. Evidence also indicates that dysphagia can occur in individuals living with Long-COVID[35,36], with up to 27% of such individuals demonstrating silent aspiration on videofluoroscopy post-COVID infection, with higher levels of intubation during the acute infection phase associated with higher levels of penetration and/or aspiration post-COVID[38].

Multidisciplinary management of Long-COVID associated communication and swallowing difficulties

Long-COVID is a new and emerging condition which currently lacks an agreed definition or clear pathway of intervention[28]. Long-COVID is described as a multi-system syndrome and requires multidisciplinary team (MDT) management to optimise patient recovery[32]. The National Institute for Health and Care Excellence (NICE) guidelines[28] recognise the role of a range of professionals in the overall rehabilitation of Long-COVID patients, advocating for the adoption of a holistic and whole-patient perspective[39]. However, regarding Long-COVID related communication and swallowing difficulties, there are no clear evidence-based MDT management guidelines. Although it is recognised that all individuals with a communication or swallowing impairment have the right to receive "timely, individual, person-centred rehabilitation, which will support and maximise

their mental health and wellbeing, participation in society, and ability to return to work"[40], SLTs have not been consistently included in the multidisciplinary management of patients living with Long-COVID even though there is an urgent need for education of MDT members in communication and swallowing difficulties in Long-COVID to optimise patient outcomes and recovery[17,41]. There is also a lack of research specifically focused on the roles and levels of training and knowledge of MDT members in the management of communication or swallowing difficulties experienced by people with Long-COVID, services may not be facilitating patients' full recovery from complications. Therefore, the aim of this research was to determine the attitudes and preparedness of healthcare workers regarding the MDT management of communication and swallowing difficulties associated with Long-COVID.

Methods

A mixed methods cross-sectional anonymous online survey was conducted in accordance with the CROSS guidelines and convenience sampling was used to investigate the attitudes and preparedness of healthcare workers regarding the management of communication and swallowing difficulties associated with Long-COVID[42]. Ethical approval was obtained from the research ethics committee from the School of Linguistic, Speech and Communication Sciences, Trinity College Dublin (TT56).

Individuals were eligible to participate if they were qualified healthcare professionals who have worked with people living with Long-COVID. Individuals were excluded if they did not have a sufficient level of English to complete the study, if they did not work with people living with Long-COVID, if they were not a healthcare worker, and if they were healthcare professionals who were not yet fully qualified (e.g. students).

Administrators working within international professional organisations, registration bodies, or support/special interest groups for healthcare workers across allied health, medical, and nursing professions acted as Gatekeepers. The healthcare organisations that were invited to facilitate this research were located internationally, including Ireland, the UK, Europe, Australia, New Zealand, the US, Canada, South Africa and India. Gatekeepers shared information regarding the survey and its purpose. In addition, Gatekeepers were provided with a link and QR code to access the participant information leaflet and the survey and they were requested to share these study details and the survey with the members of their organisation via email.

The draft survey was piloted with 3 SLT students and was adapted according to the feedback given by the pilot study participants. The anonymous survey, which contained 29 questions, was designed and disseminated using the survey software programme Qualtrics and the data collected included demographics, participants' level of clinical experiences, knowledge regarding the communication and swallowing difficulties of people living with Long-COVID and participants' perceptions of their preparedness to work with this cohort. Descriptive statistics were used to analyse the data collected.

Results

In total, 76 healthcare workers indicated interest and completed some sections of the survey, with only 27 of these individuals completing the survey in full. Therefore, these 27 fully completed surveys were included in the sample for this study **(Figure 1)**.

There were 15 (56 %) Speech and Language Therapists, 5 (18%) Physiotherapists, 4 (15%) Occupational Therapists, 2 (7%) Nurses and 1 (4%) Doctor in the sample. The levels of education of the participants varied with 13 (48%) participants reporting that they had bachelor's degrees, 13 (48%) participants reporting that they had master's degrees and 1 (3.7%) participant reporting that they had a Clinical Doctorate. The participants were working in the following countries: Ireland (n=15, 56%), Canada (n=5, 19%), America (n=4, 15%), Australia (n=2, 7%), and the UK (n=1, 3%). The clinical practice settings in which the participants worked included public hospitals (n=19, 70%), rehabilitation facilities (n=3, 12%), primary care settings (n=2, 7%), private clinics (n=2, 7%), and private hospitals (n=1, 4%). Most of the sample 81.5% (n=22) reported having clinical experience in the management of patients with Long-COVID who presented with communication and/or swallowing difficulties. A majority of the participants (56%, n=14) reported that they had not received education on the assessment and treatment of communication, or swallowing difficulties associated with Long-COVID and most of the participants (78%, n=21) reported that they would like further education in this area.

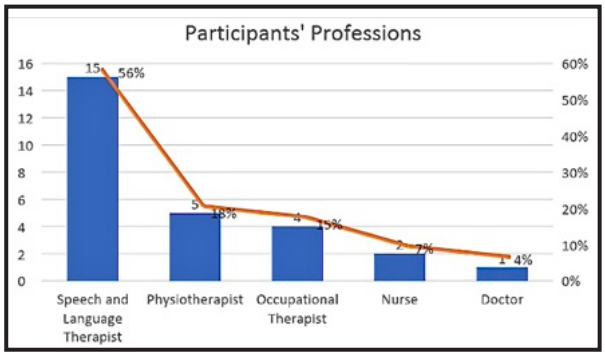


Figure 1. **Professions of participants**

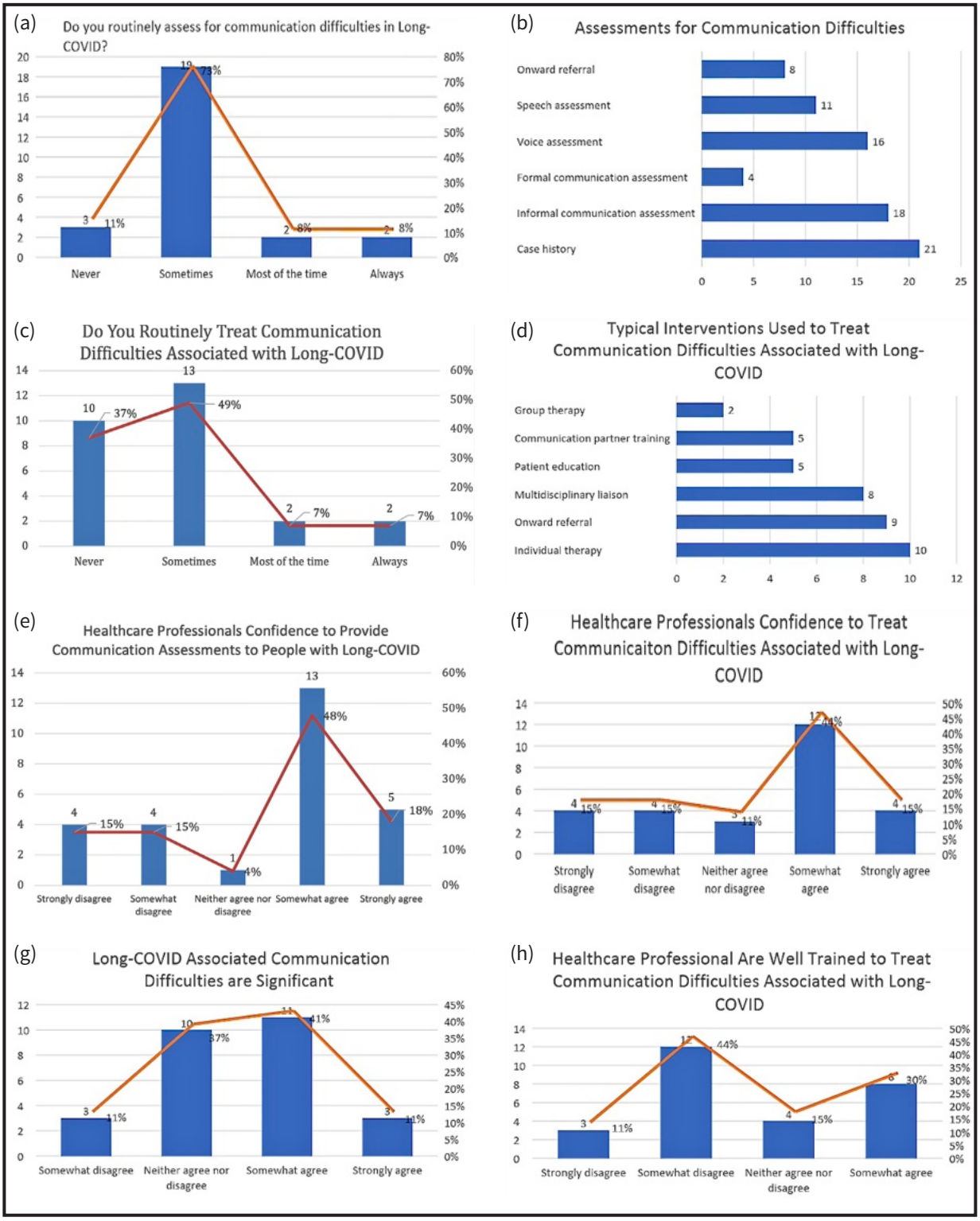
Communication Difficulties associated with Long-COVID

Assessment. Participants were asked if they assess patients for communication difficulties when they present with Long-COVID **(Figure 2)**.

Most of the participants (73%, n=19) reported that they "sometimes" assess for these issues, 11% (n=3) reported that they never routinely assess patients, 8% (n=2) reported that they assess patients most of the time and 8% (n=2) reported that they always assess this cohort of patients.

A variety of assessments were reported as routinely used to assess communication difficulties in Long-COVID **(Figure 3)**.

The most frequently used assessments to assess



(a) Figure 2. **Assessment for communication difficulties in people living with Long-COVID** (b) Figure 3. **Assessments routinely used to assess communication difficulties in Long-COVID** (c) Figure 4. **Participant responses to the question "Do you routinely treat communication difficulties associated with Long-Covid?"** (d) Figure 5. **Interventions typically used by healthcare professionals in the treatment of communication difficulties in patients with Long-COVID** (e) Figure 6. **Healthcare professionals' confidence to provide communication assessments to people with Long-COVID** (f) Figure 7. **Confidence in providing communication treatment for people with Long-COVID** (g) Figure 8. **Level of agreeance that communication difficulties are significant in Long-COVID** (h) Figure 9. **Level of agreeance that healthcare professionals are well trained to treat people with Long-COVID and communication difficulties**

Long-COVID patients for communication difficulties were case histories (n=21), informal communication assessments (n=18) and voice assessments (n=16). Some of the participants also reported that they carried speech assessments (n=11) and formal assessments.

Treatment. Participants were also asked if they routinely treat communication difficulties associated with Long-COVID **(Figure 4)**.

Most of the participants reported that they "sometimes" (49%, n=13) or "never" (37%, n=10) treat communication difficulties.

Healthcare professionals used a variety of different interventions to treat communication difficulties that present with Long-COVID **(Figure 5)**.

The most frequently reported methods used to treat communication difficulties associated with Long-COVID include individual therapy (n=10), onward referral (n=9) and referral to multidisciplinary liaison (n=8). Other interventions used by participants included patient education (n=5), communication partner training (n=5) and group therapy (n=2).

Confidence to assess and treat communication difficulties associated with Long-COVID. Most of the participants were somewhat confident or were confident in assessing communication for people with Long-COVID. Almost half of the participants (48%, n=13) reported that they somewhat agreed that they were confident and 18% (n=5) reported that they strongly agreed that they were confident to assess this cohort of patients **(Figure 6)**.

A significant proportion of participants, however, were not confident to assess communication in Long-COVID patients with 15% (n=4) reporting that they strongly disagreed and 15% (n=4) reporting that they somewhat disagreed that they were confident to assess communication for this cohort of patients.

Levels of confidence also varied among participants regarding the provision of treatment for communication challenges associated with Long-COVID **(Figure 7)**.

Most of the participants either somewhat disagreed (44%, n=12) or disagreed (11%, n=3) with the statement that healthcare professionals are well trained to treat communication difficulties associated with Long-Covid. Only 30% (n=8) of participants somewhat agreed with this statement, 15% (n=4) reported that they neither agree nor disagree and no participants strongly agreed.

Swallowing Difficulties associated with Long-COVID

Assessment. Participants were asked if they assess patients for swallowing difficulties in Long-COVID **(Figure 10)**.

Most of the participants either strongly agreed (15%, n=4) or somewhat agreed, (44%, n=12) that they felt confident in their abilities to provide intervention for communication problems associated with Long-COVID. Levels of agreement regarding the significance of communication difficulties associated with Long-COVID varied among participants **(Figure 8)**.

Just over half of the participants either somewhat agreed (41%, n=11) or strongly agreed (11%, n=3) that communication difficulties are significant in Long-COVID. However, more than half of the participants (55%, n=15) disagreed with the statement that healthcare professionals are well-trained to treat people with Long-COVID communication difficulties **(Figure 9)**.

Most of the healthcare professionals who participated in the study reported they either "sometimes" assessed (44%, n=12), most of the time assessed (11%, n=3) or always assessed (15%, n=4) for swallowing difficulties in patients who present with Long-COVID.

A variety of assessments are routinely used to assess patients who present with Long-COVID **(Figure 11)**. The most frequently used swallowing assessments were patient case histories (n=19), clinical swallow examinations (n=16) and oro-facial examinations (n=15). Other assessment methods reported included instrumental assessment (n=8), onward referral. (n=8) and using a swallow screening tool (n=5).

Participants were asked if they routinely treat swallowing difficulties associated with Long-Covid **(Figure 12)**.

Most of the healthcare professionals who participated in the study reported that they either sometimes (41%, n=11) or never (41%, n=11) treat swallowing difficulties associated with Long-COVID.

A variety of interventions were used by the healthcare professionals who participated in the study to treat swallowing difficulties associated with Long-COVID **(Figure 13)**.

The most frequently used interventions to treat swallowing difficulties associated with Long-COVID were swallow rehabilitation (n=15) compensatory strategies (n=15) patient education (n=14) and diet modification (n=13). Multidisciplinary liaison (n=10), onward referral (n=9) and medication adjustment (n=6) were also reported as interventions used to address swallowing difficulties.

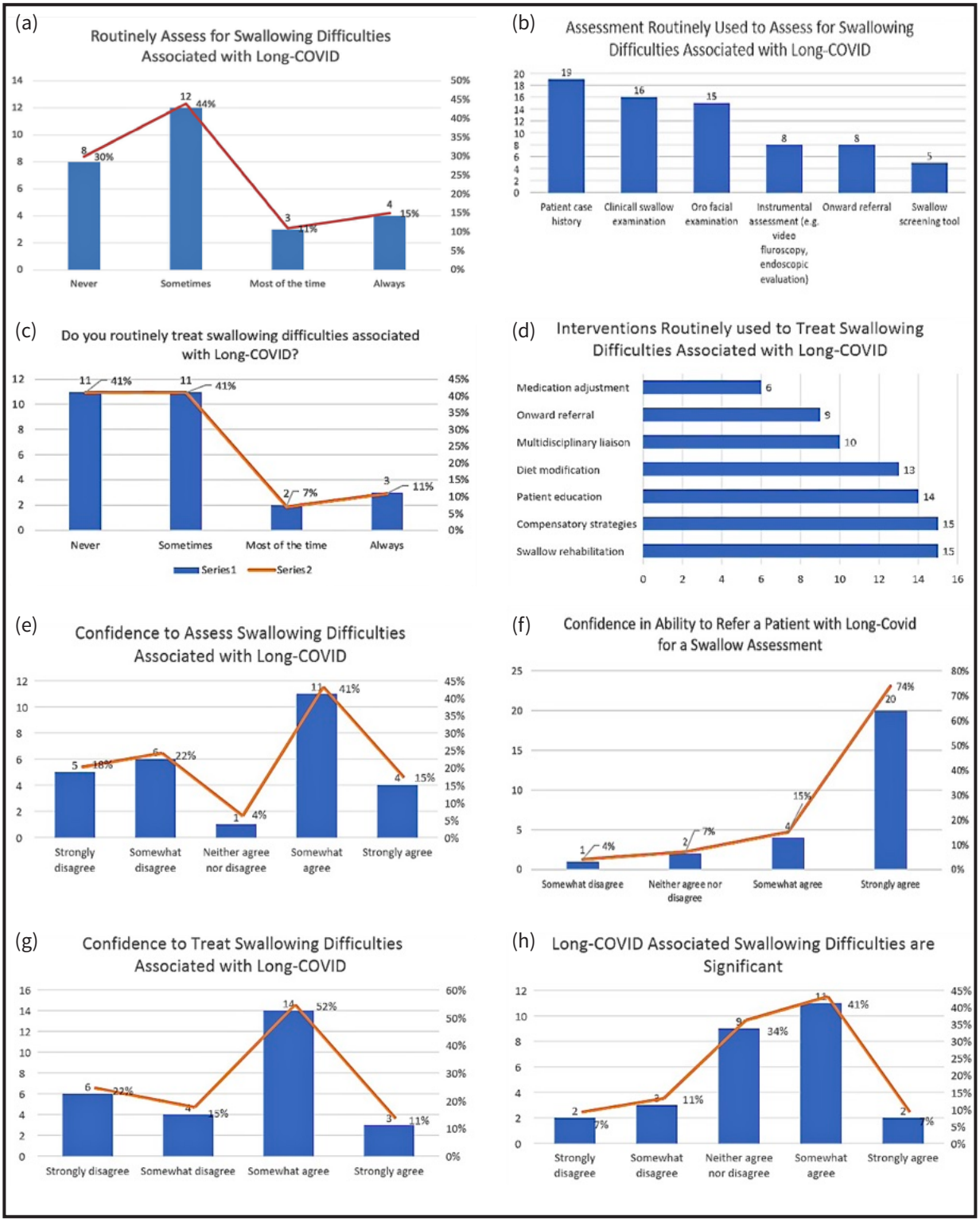
Confidence to assess and treat swallowing difficulties associated with Long-COVID. More than half of the participants (56%, n=15) reported that they felt confident in assessing swallowing difficulties associated with Long-COVID **(Figure 14)**.

Many of the participants (40%, n=10), that they either somewhat disagreed (22%, n=6) or strongly disagreed (18%, n=5) that healthcare professionals are confident to conduct swallowing assessments with patients with Long-COVID.

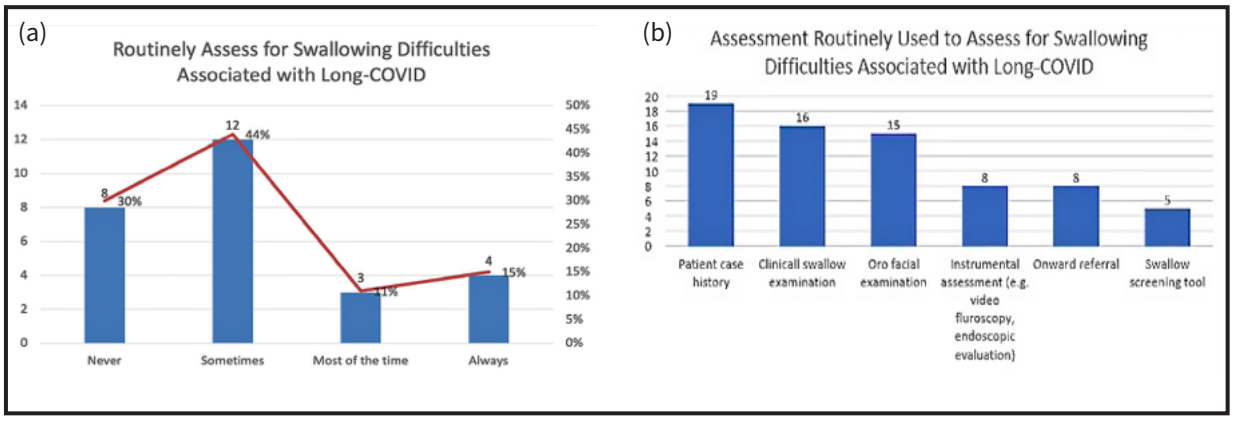
Most of the participants (89%, n=24) agreed to some degree that they felt confident in their abilities to refer a patient with Long-COVID for a swallow assessment **(Figure 15)**.

Only one (4%) participant somewhat disagreed that they felt confident to refer a patient with Long-Covid for a swallow assessment, with 2 (7%) participants neither agreeing or disagreeing, 4 (15%) participants somewhat agreeing and 20 (74%) participants strongly agreeing that they felt confident to refer a patient for a swallow assessment.

More than half of the participants 63% (n=17) agreed that they felt confident in providing intervention for



(a) Figure 10. **Routinely provide assessment for swallowing difficulties in people with Long-COVID** (b) Figure 11. **Assessments routinely used to assess swallowing difficulties in those living with Long-COVID** (c) Figure 12. **Participant responses to the question "Do you routinely treat swallowing difficulties associated with Long-Covid?"** (d) Figure 13. **Intervention typically used by healthcare professionals in the treatment of swallowing difficulties in Long-COVID** (e) Figure 14. **Healthcare professionals' confidence in conducting swallowing assessments with patients living with Long-COVID** (f) Figure 15. **Healthcare professionals' confidence in ability to refer a patient with Long-Covid for a swallow assessment** (g) Figure 16. **Healthcare professionals' confidence in providing intervention for swallowing problems** (h) Figure 17. **Participants' beliefs about the significance of swallowing difficulties**



(a) Figure 18. **Healthcare professionals' beliefs about the adequacy of training in Long-COVID and associated swallowing difficulties** (h) Figure 19. **Routine collaboration with MDT when managing communication and/or swallowing difficulties in patients with Long-COVID**

dysphagia in patients with Long-COVID (Figure 16).

More than a third of the participants (37%, n=10), however, responded that they either somewhat disagreed (15%, n=4) or strongly disagreed (22%, n=6) that they felt confident to provide intervention for swallowing problems associated with Long-COVID.

Participant beliefs varied regarding whether swallowing difficulties associated with Long-COVID are significant (Figure 17).

Almost half of the participants either somewhat agreed (41%, n=11) or strongly agreed (7%, n=2) that swallowing difficulties associated with Long-COVID are significant.

Participants were asked if they believed healthcare professionals are well trained to treat Long-COVID associated swallowing difficulties (Figure 18).

Most of the participants 62.96% (n=17) did not believe that healthcare professionals are well trained to treat people who had Long-COVID associated swallowing difficulties. Only 30% (n=8) of the participants either somewhat agreed (23%, n=6) or strongly agreed (7%, n=2) that healthcare professionals are well-trained to treat this cohort of patients.

Collaboration with Multidisciplinary Team. Participants were asked if they regularly collaborated with other healthcare professionals during the management of communication and/or swallowing difficulties for patients with Long-COVID (Figure 19).

Most of the participants (83%, n=25) reported that they either sometimes (63%, n=17), most of the time (19%, n=19) or always (11%, n=3) collaborate with a multidisciplinary team about the swallowing and/or communication difficulties experienced by people living with Long-COVID. Only 7%, (n=2) of the participants reported that they never collaborated with members of a multidisciplinary team when managing the communication and swallowing difficulties experienced by people with Long-COVID.

Education and Training in the Management of Communication and Swallowing Difficulties in People Living with Long-COVID. Participants were asked about the education they had received regarding the management of communication and/ or swallowing difficulties in Long-COVID. The majority of the participants 67% (n=18) had not received education on swallowing or communication difficulties associated with Long-COVID. Participants were asked to describe their education on swallowing or communication difficulties associated with Long-COVID and 55% (n=9) of the participants completed the qualitative section of the survey. Three themes were identified after a thematic analysis of the qualitative data **(Table 1)**.

Participants were asked about the education they would like to engage in education and training about the communication and/or swallowing difficulties in Long-COVID. Most of the participants (75%, n=21) indicated that they would like to engage in education and training. The following themes were identified after thematic analysis of the qualitative data from the participants (62%, n=17) who responded to open-ended question regarding the type of education and training that they would like to receive **(Table 2)**.

Discussion

This cross-sectional survey explored the attitudes and preparedness of healthcare workers regarding the management of communication and swallowing difficulties associated with Long-COVID. Previous literature has shown that up to 80% of those who contracted COVID-19 may develop Long-COVID, with an estimated 5 million people globally who are at risk of contracting Long-COVID[30,31]. However, this study demonstrated that 43% of participants reported no experience in working with individuals with Long-COVID in general. Yet given these high prevalence figures, it is likely that most healthcare professionals will encounter an individual living with Long-COVID within their overall caseload.

Table 1. **Qualitative themes regarding education received in relation to the management of communication and/or swallowing difficulties in Long-COVID**

Theme regarding education and training received	Explanation
Education received on the topic of acute COVID-19 infection	Participants (n=3) e reported that they have received education on acute COVID-19 and speech and/or communication difficulties.
Education received on the topic of Long-COVID via webinars	Participants (n=5) reported that they have attended webinars on Long-COVID in general.
Education received on the topic of Long-COVID was self-directed	Participants reported (n=2) that their education on swallowing and/or communication difficulties was obtained through self-directed education (e.g. via the review and analysis of evidence-based articles).

Table 2. **Qualitative themes regarding how healthcare professionals would like to upskill in the area of communication and/or swallowing difficulties in Long-COVID**

Theme regarding education and training participants would like to receive	Explanation
The wish to attend additional courses, workshops, or webinars on this topic	Participants (n=9) reported that they would like to attend webinars, courses, or workshops to upskill in this area.
The need to receive more education on screening for communication and swallowing difficulties in Long-COVID	Participants (n=4) reported that they would like to upskill in this area with a focus on education on screening for communication and swallowing difficulties in Long-COVID. Participant 15 would like education in providing "*general advice for patients awaiting SLT assessment for dysphagia.*"
The need for SLT upskilling and increased SLT staffing	Participants (n=2) reported that SLT's should be routinely involved in the management of Long-COVID, with one participant stating that their clinic should "*employ an SLT*" and that "*OTs could help upskilling SLT's on fatigue as a factor*."

Therefore, it is essential that awareness regarding Long-COVID is addressed to improve clinical interactions. More specifically, despite emerging evidence regarding the potential for communication and swallowing problems in those living with Long-COVID, most healthcare professionals reported that they either do not or that they only sometimes assess for swallowing problems in this group. Furthermore, the majority of healthcare professionals sampled here (55%, n=15) who work with people living with Long-COVID reported that they do not believe that healthcare professionals are adequately trained on the management of these concerns. As such, professionals may face clinical uncertainty when working with this group which may lead to dissatisfaction with care provided and suboptimal patient outcomes.

Attitudes regarding communication and swallowing difficulties among those living with Long-COVID

Previous research indicates that cognitive-linguistic difficulties, voice disorders, and dysphagia are common symptoms among those living with Long-COVID[16,17,20,21,34–36,43]. Bolstering these findings, the current study found that 51% and 40% of professionals here believe communication and swallowing difficulties respectively to be significant issues for those living with Long-COVID. This is an important finding as it indicates that many clinicians may be sensitive to the key concerns of patients, indicating the potential for joint goal sharing and treatment optimisation. With regards to communication specifically, recent qualitative studies have outlined the impact of Long-COVID on communication, indicating that previously skilled communicators are avoiding conversations and changing how they interact with others due to cognitive-linguistic difficulties associated with Long-COVID[17]. These findings have also been further supported by quantitative results that demonstrate that individuals living with Long-COVID perform worse than the control groups across multiple language assessments including immediate recall, delayed recall, letter fluency and narrative[16]. Although research on this topic is still limited, swallowing is also reported to be impacted, with loss of taste, difficulties with tongue function (limited elevation and lateralisation), saliva management (xerostomia, decrease of saliva in the mouth, thick saliva) and anterior spillage reported by those living with Long-COVID[44]. Therefore, it is evident that a myriad of communication and swallowing areas may be impacted, re-emphasising the need for collaborative work between patients and clinicians to advance care delivery and outcomes in this field.

Preparedness of clinicians to manage communication difficulties among those living with Long-COVID

This study discovered that the assessments used by healthcare professionals for communication difficulties in Long-COVID typically include patient case histories, informal communication, voice, or speech assessments, formal communication assessments and the C19-YRS[45]. Interestingly, formal communication assessments were

only used by 4% (n=4) of healthcare professionals, with 22.22% using informal/non-standardised methods. This echoes recent research suggesting that Long-COVID related communication concerns may not be adequately detected via traditional standardised assessments16. As many existing formal assessments are standardised on populations which don't include those living with Long-COVID, it is advised that professionals use existing formal assessments with caution, until future research recognises these individuals within their sampling processes.

Furthermore, this study demonstrates that healthcare professionals typically use a combined approach of multiple interventions in the treatment of communication difficulties in Long-COVID. These include individual or group therapy, communication partner training, patient education and multidisciplinary liaison, all of which are in line with the generalised NICE Long-COVID management guidelines[28]. However, despite the existence of these guidelines, there is a scarcity of management guidelines specifically for communication issues in this cohort. Available literature regarding COVID-related communication difficulties is primarily focused on the acute stage of COVID-19[23], with minimal focus on options available for those living with longer-term implications. Therefore, it is essential that research focuses on treatment and support options for clinicians to use with this group to provide effective and satisfactory patient support.

Preparedness of clinicians to manage swallowing difficulties among those living with Long-COVID

This study found that healthcare professionals use a combination of assessments for swallowing difficulties in Long-COVID, including case histories, clinical swallow examinations, oro-facial examinations, instrumental assessments, and swallow screening tools. Approximately a fifth (22%) of participants used clinical swallow examinations, despite recent findings that clinical swallow examinations were poor indicators of aspiration in individuals living with Long-COVID[38]. This may indicate a lack of access to, awareness of, or training in the use of more sensitive instrumental assessments. This theory is supported by results here, with only 11% of professionals reporting the use of objective swallow assessments. This finding is concerning as there is an established inherent risk of silent aspiration in the Long-COVID population[38]. As such, underlying clinical risk may be missed with reliance on clinical assessments alone, thus potentially negatively influencing clinical outcomes.

Current research suggests that the management of dysphagia should be pragmatic with a whole-patient perspective including holistic support, and treatment of the patient's symptoms[39]. Additionally, as dysphagia symptoms vary between individuals, management plans should be personalised in each case. In this vein, this study concluded that when healthcare professionals do provide intervention for swallowing difficulties in people with Long-COVID, they utilise multiple and variable treatment strategies. At a broad level, current generalised Long-COVID rehabilitation guidelines advocate for the provision of patient education, compensatory strategies, and physical rehabilitation, among other strategies[28]. As such, extrapolating these guidelines to the treatment of dysphagia demonstrates that the participants of this study are positively using intervention principles which align with current recommended practice. While this is promising, further research into the efficacy and effectiveness of specific dysphagia rehabilitation options for this cohort is required to ensure optimal patient recovery and well-being.

Education regarding communication and swallowing difficulties among those living with Long-COVID

This study found that most healthcare workers who participated (62%) have not received any specific education on swallowing or communication difficulties in Long-COVID, and the majority believe that they do not have sufficient training to treat people experiencing communication (55%) or swallowing (62%) difficulties associated with Long-COVID. Those who have completed education in this area did so via attending webinars, education on acute COVID-19, and self-led education, as opposed to organised, accredited, or modularised training. Three-quarters of these participants (75%) wished to improve their knowledge and training in this field, which lines up with previous research suggesting that upskilling for MDT members in communication and swallowing difficulties in COVID-19 and Long-COVID is imminently required[17]. However, published literature to date has not indicated how healthcare workers want to receive this additional education or which topics they prioritise it focusing on. Therefore, this research has provided new insights into how healthcare professionals would like to upskill in this area by emphasising the drive for additional courses, workshops, or webinars focusing primarily on screening methods for communication and swallowing difficulties among those living with Long-COVID, thus providing guidelines for future research and education in this field.

Limitations

The primary limitation of this study was the small sample size and limited range of MDT members who participated in the survey. Despite the use of broad sampling strategies, a limited number of professionals completed the survey in full, thus potentially limiting the generalisability of findings. In addition, the sample was primarily characterised by SLTs (55%). Furthermore, some key members of the Long-COVID MDT did not participate, namely pharmacists and dietitians. As such, the views of SLTs may have been over-represented, with the exclusion of other beneficial viewpoints. Therefore, it is advised that future research samples a broader and more representative cohort of MDT professionals working with adults experiencing Long-COVID associated communication and/or swallowing problems. Furthermore, as this research highlighted a limited amount of available education for healthcare workers regarding communication and swallowing difficulties associated with Long-COVID,

it is recommended that factors hindering provision of this supplementary training are explored (e.g. a lack of funding) to understand and tackle barriers to future care developments. Finally,, involvement of public and patient representatives in future research would be beneficial in order to determine the impact of the current lack of care for this cohort on their wellness journey and recovery.

Conclusions

This study has provided an initial insight into MDT members' attitudes towards working with people living with Long-COVID-related communication and swallowing difficulties and their preparedness to provide clinical support to this group. Overall, results indicate that a sizeable cohort of healthcare professionals recruited believe that communication and swallowing difficulties are significant clinical issues, yet they do not feel as though they have received sufficient training in the management of these concerns. Avenues for future upskilling and education in this field are suggested here, in addition to directions provided for future research in order to improve clinical care delivery to this vulnerable patient group. ◄

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SYSTEMATIC REVIEW

Is Appendicectomy Becoming Obsolete: A Review

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Abstract

Introduction: Appendicectomy has long been the chosen method for treating acute uncomplicated appendicitis; recently however, there has been debate about whether antibiotic therapy can provide a similar level of treatment. The primary aim of this review is to compare the efficacy of surgical and non-surgical interventions for acute uncomplicated appendicitis.

Methods: Relevant databases were searched for systematic reviews comparing appendicectomy against antibiotic therapy for treatment of acute uncomplicated appendicitis. The primary outcome for antibiotic therapy was improvement without recurrence of acute appendicitis within a median follow-up of one year. For surgical treatment it was confirmed appendicitis at operation with no subsequent need for surgery for acute appendicitis. The secondary outcomes include percentage of patients experiencing post-treatment complications, mean C-reactive protein on admission, and mean length of hospital stay.

Results: Eight systematic reviews satisfied the inclusion criteria. Of the 1169 patients initially treated with antibiotic therapy, 759 patients (64.93%) did not need follow up treatment within one year. This was compared to a 94.17% efficacy rate in the surgical group. There was a minor difference between post-treatment complication rates in the antibiotic and surgical groups (7.26% and 16.27%, respectively). No clear difference was found between C-reactive protein and length of hospital stay.

Discussion: This analysis shows that appendicectomy has a greater efficacy than antibiotic therapy for definitive treatment of acute uncomplicated appendicitis. However, because the rate of post-treatment complications is higher in the surgical group, patients might consider antibiotic therapy as a first option.

Keywords: *Appendicitis, conservative, operative*

Introduction

Appendicitis is defined as inflammation of the vermiform appendix. Acute appendicitis (AA) carries an estimated lifetime risk of 7–8% and is one of the most common indications for emergency surgery1. The precise causes of AA are poorly understood. Several pathophysiological pathways are proposed, stemming from infection, environmental influences, genetics, hygiene, and obstruction2.

Variable location of the appendix makes AA diagnosis challenging. History, physical examination, imaging, and biomarkers are the main diagnostic criteria. Strong clinical signs for ruling in AA in adults are right lower quadrant pain, abdominal rigidity, and radiation of pain from the periumbilical region to the right lower quadrant3. Radiological imaging, including ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), aid in the diagnosis of AA3. Patients identified with acute, localized, and uncomplicated appendicitis are then eligible for appendicectomy or nonoperative treatment4.

Appendicectomy performed by open and laparoscopic surgical techniques has historically been the gold standard treatment for AA. Laparoscopic appendicectomy demonstrates both fewer incidences of wound infections as well as faster recovery periods4. However, surgery requires general anaesthesia and often an overnight hospital stay. The main benefit of surgical treatment is that appendicitis cannot recur4. Nevertheless, negative appendicectomy is possible, with the frequency of appendicectomy having a higher incidence than that of appendicitis5.

Surgery also carries the risk of postoperative complications, including surgical site infection, post-operative intra-abdominal collection, and mortality1. With the aim of avoiding surgery, there has been a recent yet controversial push toward nonoperative treatment involving analgesia and antibiotic treatment. Hospitalisation is not typically required, and there does not appear to be an associated increased risk of appendiceal rupture4. Still, nonoperative treatment carries a failure rate at one year of approximately 25–30% requiring readmission or surgery1.

Opposing benefits and risks of appendicectomy versus nonoperative treatment are presented. Given the

recent debate regarding a superior treatment option, it is of interest whether appendicectomy is challenged by nonoperative treatment in terms of efficacy, safety, and incurred patient disability, despite being the longstanding standard of treatment. The aim of this review is to analyse current literature that compares outcomes (treatment efficacy, percentage of patients with postoperative complications, mean C-reactive protein (CRP) on admission, and mean length of hospital stay) of operative and nonoperative treatment of AA in adult patients for such parameters.

Methods

The papers considered for this review were identified in a series of computerised searches across Google Scholar, Web of Science, Medline, Embase, and PubMed with the key words: appendicitis, conservative, and operative. The 72 papers identified were first screened for duplicates using EndNote's automatic function, and 21 papers were removed. Again, using EndNote software, a subsequent search across the papers for the key term "systematic review" in the titles, keywords, or abstracts was run and 25 papers were excluded. This left 26 papers to be manually screened both to ensure the software's accuracy and to ensure all reviews were relevant to the topic. Examples of reasons for exclusion were paediatric-focused reviews and reviews focusing on complicated appendicitis. Ultimately, the final selection included eight systematic reviews.

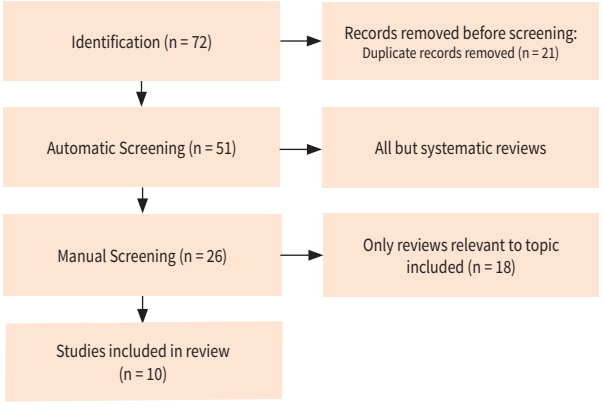

Figure 1. **PRISMA Diagram**

Results

Each systematic review we analysed referenced at least 4 randomised control trials (RCTs), as well as a variable number of retrospective cohort studies and prospective cohort studies. There was a significant recurrence of RCTs between the reviews and therefore each RCT was analysed independently using data collaboratively elucidated from the 8 systematic reviews as shown in Table 15,7,11,13–15,17,18. Thus, all the data represented in Table 1 was obtained from the systematic reviews alone.

Only the RCTs and one prospective population study present in the reviews were analysed as they contained evidence of a higher order than prospective and retrospective studies6. One RCT in a systematic review was excluded from analysis as it was retracted7.

In the studies included, diagnosis of appendicitis was decided from one or a combination of the following: clinical signs, AA history, and radiological signs. If complicated appendicitis was clinically suspected, these patients were excluded.

Our review contains data from 1983 patients above 18 years of age, with a mean age of 32.97 in the antibiotic group and 35.17 in the surgical group (Table 1).

The primary outcome our review analysed was treatment efficacy. Efficacy for antibiotic treatment was defined as definite improvement without the need for readmission for AA within a median follow-up of 1 year. Efficacy for surgical treatment was confirmed appendicitis at operation without the subsequent need for surgery for AA. The mean percentage of patients who underwent effective treatment was determined to be 63.62% for the antibiotic group and 94.17% for the surgical group (Table 1).

Secondary outcomes evaluated include the percentage of patients experiencing post-treatment complications, mean CRP on admission, and mean length of hospital stay. The mean percentage of patients experiencing post-treatment complications was 7.26% for the antibiotic group and 16.27% for the surgical group (Table 1). Our complications category included both major and minor complications ranging from wound infection to bowel obstruction. One potential limitation for complications is that most of the reviews in our study did not consider subsequent admission for recurrence of appendicectomy in the antibiotic group to be a complication; rather they considered it a failure in efficacy. The broad range of complications was also a potential source of bias and may not illustrate the complexities of each treatment method.

No large difference was found for mean CRP on admission (Table 1), although this figure was not available for two RCTs8,9. There was also no pronounced difference for mean length of hospital stay (Table 1); data was not available for one RCT in this category8

Discussion

This study has conducted a systematic review and meta-analysis to compare the efficacy (i.e., recurrence of appendicitis within one year), percentage of post-treatment complication, and mean duration hospital stay between surgical and non-surgical management of AA. In regard to efficacy, conservative treatment of appendicitis with antibiotics had an average efficacy of 63.62% compared to 94.17% for surgical treatment of appendicitis (Table 1). The disparity in the results suggest that surgical treatment should be the preferred choice as there is a much lower recurrence of appendicitis. This was similar to previous findings, where the recurrence of appendicitis decreased following an appendicectomy10. In comparison, conservative treatment with antibiotics results in the treatment of an inflamed appendix but not its removal. The appendix is therefore susceptible to infection, tumour, or faecal matter blockade, which may result in appendicitis recurrence7.

Table 1. **Studies included in the review**

Title	Authors	Date	Level of Incidence	Number in Group A/B	Mean age (A/B) Years	Male: Female A/B	Treatment Efficacy	Percentage Experiencing Post-Treatment Complications A/B (%)	Mean CRP Concentration on Admission A/B (mg/L)	Mean Hospitals Stay A/B (Days)	Appendicectomy: Open/ Laparoscopic
Randomised controlled trial of appendicectomy versus antibiotic therapy for acute appendicitis	Eriksson & Granström	1995	1B	20.0/20.0	27.8/35.0	14:6/13:7	60.0/85.0	0.0/10.0	41.0/40.0	3.1/3.4	20.0/0.0
Appendicectomy versus antibiotic treatment in acute appendicitis. A prospective multicentre randomised controlled trial	Styrud et al.	2006	1B	128.0/124.0	34.0/34.0	128:0/124:0	75.8/96.8	3.1/13.7	55.0/54.0	3.0/2.6	116.0/8.0
Randomised clinical trial of Antibiotic Therapy versus appendicectomy as primary treatment of acute appendicitis in unselected patients	Hanssone et al.	2009	1B	202.0/167.0	38.0/38.0	103:0/92:75	41.1/85.0	25.2/32.9	55.0/54.0	3.0/3.0	NS: LA + OA
Antibiotic therapy vs appendicectomy for treatment for treatment of uncomplicated acute appendicitis	Salminen et al.	2015	1B	257.0/273.0	33.0/35.0 (median)	155:102/174:99	72.8/100.0	2.3/22.3	29.0/36.0	3.2/2.8	257.0/175.0
Amoxicillin plus clavulanix acid versus appendicectomy for treatment of acute uncomplicated appendicitis: an open-label, non-inferiority, randomised controlled trial	Vons et al.	2011	1B	120.0/119.0	31.0/34.0	73.5/70.5	63.3/100.0	10.0/2.5	NS	NS	41.0/78.0
Antibiotics as first-line therapy for acute appendicitis: evidence for a change in clinical practice	Hansson et al.	2012	2B	442.0/111.0	34.0/35.0	229:218/58:53	68.8/98.2	2.9/16.2	NS	2.3/2.9	NS
Mean					**32.97/35.2**		**63.6/94.2**	**7.3/16.3**	**45.0/46.0**	**2.9/2.9**	
Total				**1983.0**							**434.0/101.0**

A: Antibiotics B:Surgical Group

When analysing post-treatment complication, the surgical group had a 16.27% complication rate, more than double compared to the antibiotic group (7.26%) (Table 1). Appendicectomy has generally been considered as the first line approach to AA. However, conflicting evidence for long term complications suggests that there might be more research needed11. Additionally, emergency appendectomy for AA, performed in instances such as bowel perforation, may cause other complications such as unplanned bowel resection (i.e., ileocecal resection or right hemicolectomy)12.

There was no substantial difference in duration of hospital stay between antibiotic treatment and surgical treatment, 2.92 and 2.94 days respectively (Table 1). This can be attributed to relatively short recovery for laparoscopic appendicectomy as well as monitoring of antibiotic treatment13.

Limitations of the study include unknown surgical methods performed with the studies selected. There was no clarification on whether open or laparoscopic appendicectomy was performed. Type of surgery is an important consideration as laparoscopic appendicectomy is a more accurate representation of the surgical treatment currently provided14.

The results we considered were only from studies that compared surgical and antibiotic treatment together. All other studies were not considered. Future research should focus on the incidence of major and minor complications between surgical and non-surgical groups in AA. Additionally, all the studies in this review included more males than females, potentially creating a source of bias. Future studies should include a more equal representation between sexes. We suggest further research in comparing management of AA in different areas of the world (resource poor vs resource rich settings) be considered.

Conclusion

This study provides arguments for both conservative and surgical treatment of appendicitis as primary treatment options. Patients looking to avoid appendicectomy should be advised that antibiotic treatment of appendicitis is a safe choice but does result in lower efficacy compared to surgical treatment. However, patients must also be informed that surgical treatment poses a higher complication rate as compared to the conservative treatment. An interesting note to highlight is that the decision to use surgical vs non-surgical management also depends on clinician judgement, experience, and resources available. Lastly, patients must be aware that recurrence of AA may occur in post-antibiotic treatment. While this study provides evidence to suggest that conservative treatment with antibiotics is safe and effective, more well-constructed studies are still required to establish the most optimal treatment for appendicitis. ◄

Declarations

There are no conflicting interests.

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SYSTEMATIC REVIEW

Systematic Review: Newer Perspective in the Medical Management of Acute Ischaemic Stroke

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Abstract

Introduction: Acute ischaemic stroke (AIS) remains a leading cause of mortality worldwide, where timely management is crucial in minimising neurological damage. This study reviews the latest evidence concerning the medical management AIS.

Methods: A systematic literature review was conducted using the EMBASE database to explore medical management strategies for AIS. Systematic reviews and meta-analyses from the past five years underwent abstract screening by two reviewers. Eligible abstracts were then evaluated through full-text reading based on the eligibility criteria. Out of 174 identified citations, 21 studies were included in the narrative analysis.

Results: Current evidence supports alteplase as the first-line treatment for AIS. However, tenecteplase is emerging as a promising alternative with similar efficacy and lower risks. Recent clinical trials suggested the use of aspirin within 24 hours of onset, and the consideration of dual antiplatelet therapy for prevention. Routine use of anticoagulants is discouraged, but low molecular weight heparin may have a role in certain stroke types. Emerging neuroprotective agents like edaravone, minocycline, vinpocetine, and salvianolic acids show promise but require more research for inclusion in treatment guidelines.

Conclusion: Medical management of AIS primarily relies on alteplase as the gold-standard treatment. Our analysis highlights tenectaplase as a possible alternative as well as the potential benefits of antithrombotic agents and neuroprotectants for post-stroke recovery and prevention. With variations in clinical efficacy outcomes due to limited subgroup analyses, it is important to conduct large multicentre trials to further evaluate the various management strategies in order to establish optimal care which is personalised.

Keywords: *Acute ischaemic stroke, Medical management, Alteplase, Antithrombotic agents, Neuroprotectants*

Introduction

Acute ischaemic stroke (AIS) is characterised by an interruption of blood circulation in the brain, limiting blood and oxygen delivery[1]. Stroke is the second leading cause of mortality worldwide, with approximately 12 million individuals suffering from an ischaemic stroke annually[2]. Stroke incidents can be classified as either ischaemic, which accounting for about 87% of all cases, or haemorrhagic, caused by a rupture of a blood vessel in the brain[3]. The underlying pathophysiology of ischemic stroke is most commonly due to thrombosis, typically of cardio-embolic origin secondary to atrial fibrillation or other arrhythmias[3]. Other less common causes include large-vessel stenosis, small-vessel disease, and infective vegetations[3].

Stroke is a medical emergency with severe consequences to the patient's quality of life. Despite current medical management, 60-80% of patients face either mortality or a lack of full recovery within 90 days following infarction[4]. The management of anterior circulation stroke has been shown to require a multidisciplinary approach and is in constant evolution[5]. 75-80% of strokes occur in the anterior circulation, with the rest occurring in the posterior circulation[6]. Currently, the gold standard treatment for AIS is the thrombolytic agent alteplase, which dissolves the clots in order to restore brain perfusion. Additionally, mechanical thrombectomy, an interventional approach that removes the clots using a catheter, is often considered[7]. Although effective, thrombectomy carries a 15% risk of complications, including arterial perforation and post-operative haemorrhage, which can be life-threatening[8]. Due to the high-risk nature of invasive procedures, it is crucial to optimise medical management and continue efforts to discover new pharmacological agents for AIS treatment.

An evidence-based approach is required for a high standard of medical care, especially when considering the introduction of novel therapeutics. Therefore, the

aim of this review is to evaluate the current evidence-based medical management of acute ischaemic strokes.

Method

Database search

A systematic review database search was conducted on October 25th, 2022 via the EMBASE database. The search was limited to the last 5 years to focus on the evidence and justification of the current management strategies for acute ischaemic stroke. The aim for this review was to evaluate the current medical management strategies for acute ischaemic stroke, therefore, the keywords used were 'medical management' or 'management' and 'acute ischaemic stroke' or 'anterior circulation stroke'. As the focus was solely on medical management, surgical management keywords such as 'thrombectomy' and 'endovascular' were removed by using the NOT function. Finally, to find literature which has high level of evidence **(Table 1)**, an exclusive search was conducted looking for 'meta analysis' or 'systematic review'. The full search strategy is as below:

('medical management'/exp OR 'medical management' OR 'management' OR (medical AND ('management'/exp OR management))) NOT 'thrombectomy' NOT 'endovascular' AND ('acute ischemic stroke'/exp OR 'acute ischemic stroke' OR 'acute ischaemic stroke'/exp OR 'acute ischaemic stroke' OR 'anterior circulation stroke'/exp OR 'anterior circulation stroke') AND ('meta analysis'/de OR 'systematic review'/de) AND ('article'/it OR 'review'/it) AND (2018:py OR 2019:py OR 2020:py OR 2021:py OR 2022)

Study selection

Screening of title and abstracts: Two reviewers screened each article by title and abstract of the retrieved search results in EMBASE based on the eligibility criteria **(Table 2)**. Any conflicts in inclusion or exclusion were discussed until a consensus was reached.

Table 1. **Levels of Evidence Grading System**[9]

Level	Type of Evidence
1a	Systematic Review (with homogeneity) of Randomised Clinical Trials (RCT)
1b	Individual RCT (with narrow confidence intervals)
1c	All or none study
2a	Systematic Review (with homogeneity) of cohort studies
2b	Individual cohort study (including low quality RCT, e.g. <80% follow-up)
2c	"Outcomes" research (i.e. audits)
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 critical appraisal

Full-text review: The included abstracts were downloaded and assessed based on their full text. Two reviewers independently screened each paper for inclusion or exclusion based on the eligibility criteria **(Table 2)**.

Results of Database Search

Database search

The EMBASE database identified 174 citations based on the eligibility criteria for this review **(Table 2)**. Seven duplicates were found, and 167 citations were moved to title and abstract screening in the systematic review management tool Covidence[10]. 100 citations were excluded based on title and abstract. Upon full-text review of 67 citations, 46 were further excluded, leaving 21 citations for inclusion in the narrative analysis. The 2020 PRISMA guidelines[11] were used for this literature review to ensure a systematic approach to searching the literature. A flow diagram of the database search methods is shown in **Figure 1.**

For each study included in this review, seven important components were extracted and presented in a table as seen in **Table 2**. The seven aspects include:

1. Name of journal and year of publication
2. Number of studies included in the systematic review/meta-analysis
3. Number of participants and their mean age
4. Grading scale of evidence
5. Type of medical management
6. Type of group comparison
7. Outcome of study

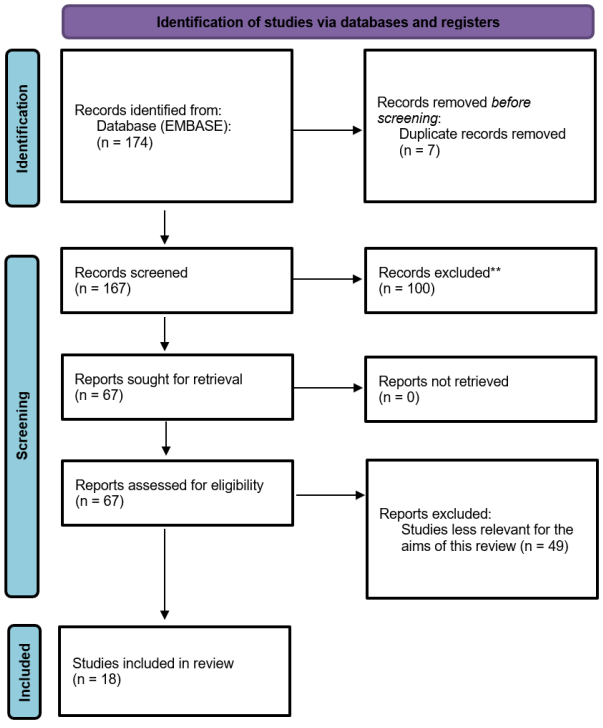

Figure 1. **PRISMA flowchart**

Table 2. **Eligibility Criteria**

	Inclusion Criteria	Exclusion Criteria
Publication year	Last 5 years (Oct 2018 – Oct 2022)	
Language	Restricted to English language	
Types of articles	Scientific articles published in peer-reviewed journals with available full texts that analyse at least one RCT	• Popular articles, grey literature • Editorials, commentaries etc. • Abstracts only • Conference abstracts • Trial registries
Study design	Systematic reviews and/or meta-analysis	• Study protocols • Clinical trials • RCTs • Case series • Case reports • Observational studies
Population	• Patients with anterior circulation stroke • Patients with acute ischaemic stroke	• Posterior circulation stroke • Haemorrhagic stroke • Comorbidities with specific medications (statins, heparin prior to intervention) • Large vessel occlusion • Specific patient populations (aspirin allergy, dyspepsia)
Interventions	Any patient targeted pharmacological approach to treating anterior circulation stroke	• Thrombectomy • Any other surgical interventions • Herbal medicine
Outcomes	• Neurological improvement • Risk of intracranial haemorrhage (ICH) • Mortality • Recurrent ischaemic stroke (RIS) post intervention	Failure of intervention

Main findings

Thrombolysis

The current guidelines for the pharmacological treatment of acute ischemic stroke (AIS) recommend the use of alteplase within 4.5 hours of onset of stroke symptoms[30]. Alteplase, a fibrinolytic agent often referred to as tissue plasminogen activator (tPA), converts plasminogen to plasmin, an enzyme that breaks down fibrin into fibrin degradation products, thereby dissolving the blood clot[31]. However, as a fibrinolytic agent, it also increases the risk of symptomatic intracerebral haemorrhage (sICH). Jia and colleagues investigated the safety and efficacy of alteplase treatment in ischaemic stroke and found that, although it resulted in better functional outcomes compared to other non-thrombolytic treatments, the risk of sICH was 4.46 times higher with alteplase use[12]. Therefore, it is crucial that these agents are administered with extra care and continued monitoring. A recent meta-analysis indicated that low-dose alteplase (<0.75mg/kg) could achieve a prognosis similar to the standard dose (0.9 mg/kg) with a reduced incidence of sICH[13]. Regarding the timing of administration, two meta-analyses concluded that the risk of sICH significantly increases beyond 4.5hrs after symptom onset, making timely administration of alteplase crucial[14, 15].

Although alteplase is the current recommended fibrinolytic agent for thrombolysis, tenecteplase is another agent that has shown promising results. Two recent meta-analyses compared the safety and efficacy of alteplase and tenecteplase[16, 17]; both studies concluded that tenecteplase demonstrated higher recanalisation rate and early neurological improvement with doses of 0.20-0.25 mg/kg. Safety considerations showed that sICH and mortality rates were comparable between both drugs. Tenecteplase also has a longer half-life and is suitable for a single bolus administration, making it preferable for patients requiring transfer for endovascular therapy. Additionally, tenecteplase has been shown to be more cost-effective, with an approximate cost of $3000 for 25mg of tenecteplase compared to $8000 for 90mg of alteplase which are appropriate dosages for a 100kg person[32]. A change to tenecteplase as the primary medication could potentially save hospitals and patients a substantial amount of money. These results strongly suggest that tenecteplase is a compelling alternative to alteplase, warranting a reassessment of The National Institute for Health and Care Excellence (NICE) guidelines to consider tenecteplase as the first-line medication for intravenous thrombolysis (IVT).

Anti-thrombotics

In addition to their prominent role in long-term secondary cardiovascular prevention, anti-platelets are also important for treating AIS. Primary antiplatelet agents in the treatment of AIS are Aspirin and Clopidogrel. Aspirin irreversibly inhibits cyclooxygenase (COX), leading to reduced thromboxane A2 (TXA2) production

and thus permanently inhibiting platelet aggregation[33]. Clopidogrel inhibits platelet aggregation through the P2Y12-receptor pathway, working synergistically with aspirin in platelet-aggregation assays[34]. NICE guidelines recommend the use of aspirin within 24 hours of AIS onset[30]. Minhas and colleagues confirmed the safety and efficacy of early antiplatelet therapy, especially within 48 hours of onset[18].

The increased risk of bleeding is offset by reductions in mortality or dependency and incidence of recurrent ischaemic stroke. Dual antiplatelet therapy (DAPT) with aspirin and clopidogrel, as opposed to aspirin alone, has been linked with a reduced risk of recurrent ischaemic stroke but increases bleeding risk, which can be minimised by limiting the duration of DAPT[19]. Physicians must balance efficacy and safety when prescribing antiplatelets, and should consider risk factors such as a history of stroke or intracranial haemorrhage[35].

According to the NICE guidelines, anticoagulants are not recommended for routine use in AIS[30]. Wang and colleagues found no benefit from early anticoagulation, with reductions in recurrent ischaemic stroke and pulmonary emboli being offset by an increased risk of sICH and extracranial haemorrhage[20]. Xia and colleagues found low molecular weight heparin (LMWH) offered no additional benefits over aspirin in an unselected patient population while increasing the risk of extracranial haemorrhage[21]; however, in patients with non-cardioembolic ischaemic stroke, LMWH reduced incidence of early neurological degeneration and recurrent ischaemic stroke while improving independence. Thus, the use of anticoagulants such as LMWH may depend on the type of ischaemic stroke and must be used with caution.

Neuroprotectants

An alternative intervention involves the use of neuroprotectants which help to reduce neurological damage following reperfusion to the ischaemic region. These novel interventions are currently not part of the NICE guidelines; however, recent promising have shown efficacy in reducing irreversible neuronal damage.

For the free-radical scavenger edavarone, Chen and colleagues found increased neurological improvement and decreased mortality rates at three months post-stroke[22]. It is thought that edavarone removes free radicals produced due to brain tissue ischaemia, leading to a reduction in oxidative stress. They concluded that edaravone has clear benefits in treating AIS and has been used within the Asian health system[22]. Hu and colleagues found similar results in their systematic review of the use of edaravone combined with alteplase, in which there was a reduction in National Institutes of Health Stroke Scale (NIHSS) scores as well as a reduced risk of ICH[23]. NIHSS is a standardised scale estimating stroke severity, ranging from 0 to 42, with higher scores indicating greater severity[36]. Moreover, Yang and colleagues found that adding a kallikrein glycoprotein neovascularisation agent (human urinary kallidinogenase) to edaravone significantly improved NIHSS scores better than edaravone alone[24]. Human urinary kallidinogenase (HUK) activates the kallikrein/kinin system (KKS) inducing angiogenesis and neovascularisation, potentially restoring blood supply in the ischaemic regions[24].

Other neuroprotectants include minocycline, a tetracycline with neuroprotective properties, which was found to reduce NIHSS scores in AIS patients after three months[25]. Vinpocetine and salvianolic acids, which are both plant derivates, similarly showed reductions in three-month NIHSS scores[26, 27]. In addition to pharmacological neuroprotectants, alternatives like remote ischaemic conditioning and normobaric oxygen therapy, when administered for three to six hours, have both been found to reduce NIHSS scores, although these studies have limited clinical use and evidence of benefit[28, 29]. These novel therapeutic approaches may continue to develop and may have potential to be added in the NICE guideline regimen for treating AIS in the future.

Discussion

The findings from recent research on the medical management of acute ischaemic stroke suggest that alteplase remains to be the gold standard and most effective treatment option; however, it should be administered with careful consideration due to the associated risk of sICH. Meanwhile, tenecteplase shows promise as a viable alternative to alteplase, as it offers similar efficacy with a potentially lower risk profile. Perhaps, a more large-scale multi-centred clinical trial is necessary to confirm whether tenecteplase should be the first line treatment instead of alteplase. The guidelines recommend antiplatelet therapy with aspirin, especially within the first 24 hours of acute ischaemic stroke onset, and dual antiplatelet therapy may be considered for recurrent ischaemic stroke prevention, although this approach should carefully balance its benefits against the risk of bleeding.

Anticoagulants are generally discouraged for routine use in AIS due to the increased risk of sICH. However, low molecular weight heparin (LMWH) might have a role in specific stroke types such as strokes of non-cardioembolic origin. Additionally, emerging neuroprotective agents like edaravone, minocycline, vinpocetine, and salvianolic acids demonstrate the potential to reduce neurological damage, but further research is needed to solidify their inclusion in treatment guidelines and understand their long-term effects. These findings emphasise the importance of tailoring AIS treatments to the individual needs of the patient while exploring possible alternatives to the standard approach.

Regarding long-term outcomes in patients treated with IVT, DAPT and neuroprotectant agents, a cohort study by Muruet et al with a ten-year follow up found that thrombolysis with intravenous alteplase is linked to better long-term survival and functional outcomes, with treated patients living on average one year longer than controls[37]. Additionally, a recent systematic review found that both short-term and long-term use of DAPT reduces risk of stroke; however, the risk of intracranial bleeding increases with DAPT use beyond three months[38]. Edaravone, a promising neuroprotective agent, has been

Table 3. **Data extraction from included papers**

Author and Year	Number of studies included	Number of participants	Mean age (years)	Grading Scale of Evidence	Medical Management	Comparison	Outcome	Reference
Sun et al., 2022	16	5,846	N/A	1a, 2a	Alteplase for mild ischaemic stroke (MIS) ("MIS patients generally do not undergo thrombolytic therapy, as clinicians generally assume these patients to have a better prognosis…)	rt-PA thrombolytic therapy vs. other non-thrombolytic treatments	• Good functional prognosis (mRS 0-1): Patients who underwent thrombolytic therapy had better outcomes at 3 months post-treatment relating to patients that underwent non-thrombolytic treatment. • Risk of sICH was 4.46 times higher in patients that underwent rt-PA treatment relating to patients that underwent non-thrombolytic treatment. • Mortality: rt-PA and non-thrombolytic treatments were not correlated with differences in mortality. • MIS cases exhibited improved 90-day favourable functional outcomes following alteplase treatment relative to controls. However, such treatment was also correlated with an enhanced chance of sICH	12
Cheng et al., 2018	12	7,686 low-dose: (n = 2888) standard-dose: (n = 4798)	N/A	1a	Low-dose tPA and normal dose tPA	Low dose tPA vs normal dose tPA	• Improved mRS scores with low-dose tPA, lowered incidence of sICH, similar effect of mortality and neurological function. • Low-dose tPA is highly recommended in AIS patients.	13
Jia et al., 2021	4	848	N/A	1a	Use of IVT (IVT group) among AIS patients with unclear symptom onset or extended time window (i.e., >4.5 hrs) Mean time window in the IVT group was 7.2 to 10.3 hrs and 7.3 to 10.4 hrs in the CG	IVT vs control group (CG)	• The functional independence at 90 days: There was a significant difference between the IVT group and the CG with the IVT group having better functional independence. • Meta-analysis of sICH found that the IVT group had higher rates than the CG and there was no significant difference between the IVT group and the CG in incidence of death. • The results of this meta-analysis confirmed that IVT was beneficial for patients with stroke lasting >4.5 h, and this treatment method can effectively improve the clinical functional outcome of patients compared with placebo	14
Chen et al., 2020	12	3,402	70	1a	Alteplase <3 hrs, Alteplase 3–4.5 hrs, Alteplase >4.5 hrs post-AIS	Alteplase vs control	• IV alteplase regardless of the time delay significantly improved the proportion of patients with modified Rankin Scale (mRS) scores 0–1 at 90 days after acute ischaemic stroke whereas IV alteplase used within 3 hours was more effective than that exceeding 3 hours. • V alteplase beyond 3 hours significantly increased the rate of symptomatic intracerebral haemorrhage within 36 hours compared with placebo but that within 3 hours was comparable with placebo.	15
Oliveira et al., 2021	8	2,031	69	1a	Various time windows ranging from <3hrs to <6 hrs Tenecteplase dosage: 0.1, 0.2, 0.25, 0.4 mg/kg	Tenecteplase vs alteplase	• Tenecteplase demonstrated an increase in recanalization rate and early neurological improvement. • No differences shown in terms of sICH, or mortality. • 0.20–0.25 mg/Kg tenecteplase dose might be preferable to other dosages as it was associated with higher early neurological improvement rates and a tendency for better functional outcome at 3 months. • The results of this study strongly suggest that tenecteplase is a valid alternative to alteplase for thrombolysis in ischemic stroke patients.	16

Table 3. **Data extraction from included papers (Cont.)**

Author and Year	Number of studies included	Number of participants	Mean age (years)	Grading Scale of Evidence	Medical Management	Comparison	Outcome	Reference
Kheiri et al., 2018	5	1,585 Tenecteplase: (n = 828) Alteplase: (n = 757)	70.8	1a	Tenecteplase dosage: 0.25mg/kg, 0.1mg/kg, or 0.4mg/kg Alteplase dosage: 0.9mg/kg	Tenecteplase vs alteplase	• Tenecteplase-treated patients were more likely to achieve complete recanalization of the occluded vascular territory and early neurological improvement. • No differences with regards to excellent recovery (mRS 0–1), functional independence (mRS 0–2), or poor recovery (mRS 4–6). • Tenecteplase-treated patients had no increased risk of symptomatic or any intracerebral haemorrhage or mortality compared with alteplase-treated patients. • Tenecteplase with a dose of 0.25 mg/kg was most effective in achieving early neurological improvement, complete and partial recanalization, and excellent mRS outcomes, without increased risks of intracerebral haemorrhage or mortality. • Study demonstrated tenecteplase's efficacy and safety profile were similar to, and sometimes even better than, alteplase in AIS patients	17
Minhas et al., 2022	11	42,262	N/A	1a	Oral antiplatelet therapy started within 14 days of stroke - aspirin (160 mg to 300 mg daily started within 48hrs of stroke symptom onset) other drugs: thienopyridine derivatives inhibiting adenosine diphosphate receptors (dipyridamole, cilostazol) and thromboxane A2 antagonists (ozagrel)	Oral antiplatelet therapy vs controls	• Death and dependency = Oral antiplatelet therapy led to a significant reduction at end of follow up, significant reduction at end of treatment period, reduction at final followup of greater than 1 month. • Oral antiplatelet therapy led to a reduction in recurrent ischaemic/unknown stroke. • Symptomatic ICH = increased odds for oral antiplatelet therapy but not statistically significant. • Any recurrent stroke/ICH = Oral antiplatelet therapy reduced odds of ischaemic stroke but also increased odds of symptomatic ICH. • Significant increase in risk of extracranial haemorrhage for oral antiplatelet therapy.	18
Yang et al., 2021	7	133,502	N/A	1a	Doses of clopidogrel and aspirin: 75mg and 325 mg once daily, respectively.	Clopidogrel and aspirin (DAPT) vs aspirin monotherapy	• Clopidogrel plus aspirin significantly lowered the risk for recurrent stroke compared with aspirin monotherapy. • All included reviews concluded that dual antiplatelet therapy (DAPT) appeared to be more effective and safer than monotherapy, and that using DAPT for as short as possible maximises benefit without increasing the risk for bleeding.	19
Wang et al., 2021	28	24,025	N/A	1a	Subcutaneous and intravenous standard unfractionated heparin, low-molecular-weight heparins, subcutaneous and intravenous heparinoids, oral vitamin K antagonists, factor Xa inhibitors, and specific thrombin inhibitors.	Anticoagulant therapy (started within two weeks of stroke onset) vs control	• Anticoagulation was associated with a statistically significant reduction in recurrent ischaemic stroke (OR 0.75, 95% CI 0.65 to 0.88; P =0.0003; 12 RCTs, 21,605 participants. • Early anticoagulation significantly increased symptomatic intracranial haemorrhage more than twofold. • Anticoagulation was associated with a significant reduction in pulmonary embolism. • Anticoagulation was associated with a significant increase in major extracranial haemorrhage. • Anticoagulation was associated with a significant reduction of deep vein thrombosis, although a majority of deep vein thromboses detected were subclinical and asymptomatic.	20

Table 3. **Data extraction from included papers (Cont.)**

Author and Year	Number of studies included	Number of participants	Mean age (years)	Grading Scale of Evidence	Medical Management	Comparison	Outcome	Reference
Xia et al., 2022	5	4,625	N/A	1a	Low molecular weight heparin (LMWH) vs. aspirin for early management (within 14 days of onset)	LMWH vs Aspirin	• Recurrent ischaemic stroke (RIS): No significant difference between LMWH and aspirin (RR: 1.02, 95% CI: 0.74–1.39). • Independence (mRS of 0-2): All five RCTs reported an mRS score of 0–2 at the end of follow-up (3 months for one study and 6 months for remainder); no difference between LMWH and aspirin (RR: 1.00, 95% CI: 0.95–1.06). • Death from any cause during treatment period and at the end of follow-up: No significant difference between LMWH and aspirin both in the treatment period (RR: 1.14, 95% CI: 0.97–1.27) and at the end of follow-up (RR: 1.01, 95% CI: 0.92–1.10). • Symptomatic intracranial haemorrhage during treatment period: No difference between LMWH and aspirin (RR: 1.19, 95% CI: 0.95–1.49). • LMWH was significantly associated with extracranial haemorrhage (RR: 1.16, 95% CI: 1.04–1.29; I2 = 37.9%, P=0.168).	21
Chen et al., 2021	7	2,069	70.1	1a	Three RCTs investigated patients with a treatment time window within 24 h, and three RCTs investigated patients with stable vital signs who were admitted to hospitals during 24-72 hours after the onset of stroke. Five RCTs investigated patients treated with edaravone for two weeks and the other two RCTs included patients treated with edaravone for one week. Four RCTs evaluated outcome at the long-term follow-up, namely three months or later. The other three RCTs made an assessment at two weeks follow-up.	Comparison of edaravone plus conventional therapy vs routine treatment alone Edaravone vs placebo or no intervention	• Three RCTs including 1720 patients reported the mortality at three months follow-up → a significant reduction of mortality was observed in the edaravone group than in the control group (RR = 0.55, 95% CI, 0.43- 0.70, I 2 = 0%; p < 0.01). • Four RCTs including 1778 patients evaluated the improvement of neurological impairment according to the authors' judgements at three-month follow-up → a significant improvement of neurological impairment was observed in the edaravone group than in the control group (RR = 1.54, 95% CI, 1.27-1.87, I 2 = 0%; P < 0.01). • Four RCTs including 466 patients reported the incidence of adverse events during the treatment → there was no significant difference in the incidence of adverse reactions between two groups (RR = 0.83, 95% CI: 0.51-1.34, P = 0.43) including nausea, skin rash, and abnormal liver function.	22
Hu et al., 2021	17	1,877 Edaravone: (n = 939) No Edaravone: (n = 938)	N/A	1a	Edaravone dosage: 60 mg/day	rt-PA combined with edaravone vs control	• 15 studies showed that rt-PA combined with edaravone treatment was associated with a reduced NIHSS score. • Eight studies with 946 patients reported ICH. Five studies indicated that edaravone treatment could not reduce the rate of intracranial haemorrhage, while another three studies reported a significant reduction of ICH in the edaravone group. • Pooled analysis found that edaravone treatment was associated with a lower risk of ICH • Four studies involving 442 patients: Pooled analysis demonstrated that no significant relationship between edaravone treatment and mortality could be shown.	23

Table 3. **Data extraction from included papers (Cont.)**

Author and Year	Number of studies included	Number of participants	Mean age (years)	Grading Scale of Evidence	Medical Management	Comparison	Outcome	Reference
Yang et al., 2021	13	1,242	50+	2b	Human urinary kallidinogenase (HUK) dosage: 0.15 PNA/day Edaravone dosage: 30 mg/day The mean values for clinical outcomes were assessed at 14 days.	HUK and edaravone vs edaravone alone	• The random-effect model revealed that the NIHSS score of patients treated with HUK combined with edaravone was lower than that of patients treated with edaravone alone, and the difference was statistically significant (WMD = −3.92, 95% CI (−4.82, −3.02), p < .0001), with high evidence of the heterogeneity(I2 = 98.0%, p < .001). • The result of pooled effect by fixed-effect model showed that compared with edavarone alone group, the ADL score of the HUK combined with edavarone group was significantly higher (WMD = 14.13, 95% CI (10.67, 17.60), p < .0001), with considerable heterogeneity among studies (I2 = 93.8%, p < .0001).	24
Malhotra et al., 2018	7	426	N/A	1a	Minocycline alongside standard treatment (tPA)	Minocycline + standard treatment vs standard treatment	Minocycline is a safe and efficacious agent in the treatment of acute ischemic strokes	25
Panda et al., 2022	4	837	62.4	1a	Vinpocetine started within 14 days of AIS Median dose: 30mg/day	Vinpocetine + standard treatment vs standard treatment alone)	• Vinpocetine has some promising efficacy in patients with ischemic stroke when used in the acute stage in reducing the disability, but presently there is not enough evidence to suggest it also reduces case fatality. • More double-blind, placebo-controlled RCTs of adequate sample size are needed before making recommendations for the routine administration of vinpocetine for all patients with acute ischemic stroke.	26
Xin et al., 2020	58	5,309	N/A	1a	Salvianolic acid (SA) treatment alongside standard western medical treatment (tPA, antiplatelet therapy, cerebral protectants)	SA + standard treatment vs standard treatment	• SA can significantly improve the total clinical effectiveness rate of ACI patients. • The use of SA remarkably increased the neurological functions, short-term daily living ability recovery, and cognitive functions of ACI patients	27
Zhao et al., 2018	7	735	N/A	1a	Remote ischaemic conditioning (RIC) (for prevention and treatment of ischaemic stroke)	RIC vs. non-RIC or standard medical management alone	• RIC did not significantly reduce the final infarct volume in people with acute ischaemic stroke who received intravenous thrombolysis, but it might increase the death or dependency of these people. • Neurological impairment and psychological impairment were not significantly improved by RIC in this patient population.	28
Bo et al., 2019	6	292	N/A	1a	Normobaric Oxygen (NBO) Oxygen therapy time window (<20 min, 20-120 mins, >120 mins); Oxygen concentration (29%, 45%, 61%); Air circulation (none, 3hrs NBO + 2hrs air, 6hrs NBO + 2 hrs air); Duration (24hrs, 48hrs, 72hrs)	NBO vs control groups	• NBO induced greater improvement in neurological recovery after stroke. • Improved ADL and disability of patients. • No effect on recurrence rate and mortality of AIS	29

Abbreviations: mRS = modified rankin scale, sICH = symptomatic intracranial haemorrhage, rt-PA = recombinant tissue plasminogen activator, IVT = intravenous thrombolysis, AIS = acute ischaemic stroke, CG = control group, DAPT = dual antiplatelet therapy, ADL = activities of daily living, NBO = Normobaric Oxygen, RIC = remote ischaemic conditioning, SA = salvianolic acid, ACI = acute cerebral infarction

shown to improve daily activities, reduce neurological deficits, and increase recanalisation rates in the short term[39]. However, further research and validation through larger clinical trials are necessary to ascertain its long-term effects.

The majority of our findings were derived from systematic reviews of randomised control trials (RCTs) which are considered to be the gold standard in evaluating the efficacy of health interventions. Furthermore, the method and search strategy in this paper allowed for investigation into a variety of medical management strategies of acute ischaemic stroke, including the most efficacious pharmacological agent to use for AIS in the general population[14-17, 30] and the role of newer agents like neuroprotectants[22-29]. Furthermore, we integrated two network meta-analyses, which, in contrast to conventional pair-wise meta-analysis, allows for the generation of multiple pooled effect estimates. This enables the ranking of interventions based on efficacy for example the different time points in which alteplase can be administered[15,17]. This review also included studies published in non-English languages, which included studies from all over the world which reduces the impact of language and culture bias on the total estimate of treatment effectiveness.

However, there are several limitations of the studies included in our analysis. There are high levels of heterogeneity in several clinical outcomes observed in the papers discussing the efficacy of different pharmacological agents in AIS. Most of the included studies' analyses failed to separate the percentage of people with certain stroke subtypes and stroke aetiologies, which may have had an impact on how well participants respond to thrombolytic agents. Data collection on neuroprotective agents was challenging due to limitations in the studies such as small homogenous populations (entirely Chinese cohorts), which may limit generalisability. Furthermore, in several instances, the methodological design of the studies was described as improper or incomplete, oftentimes not including long-term outcomes at six months or a year. Therefore, it was difficult to state whether the medical management had a long-term significant effects on the cohort.

Another notable limitation of the studies included in our analysis is the absence of subgroup analysis examining the potential impact of sex and ethnicity on the efficacy of thrombolytic agents, antiplatelet agents and neuroprotectants. A recent systematic review and meta-analysis by Liu et al. found that female stroke patients treated with IVT were more likely to exhibit worse functional outcomes compared to males, although the safety profile regarding haemorrhagic complications from was similar between sexes[40]. Additionally, a study by Mehta et al. identified potential differences in the side-effect profile of thrombolysis linked to the patient's ethnicity[41]. The incidence of sICH was slightly higher in patients of African or Afro-Carribean and Asian ethnicity in comparison to Caucasian patients[41]. Further research is required to explore sex- and ethnicity-linked differences in outcomes of thrombolysis and stroke management in order to develop personalised treatment strategies.

Conclusion

Medical management of AIS remains a first-line treatment, with alteplase recommended as the gold-standard treatment. Our analysis also indicates that antithrombotic agents and neuroprotectants have potential advantages in promoting functional recovery post-stroke as well as secondary prevention. However, only a small percentage of the reviews we evaluated included a subgroup analysis which can lead to varying clinical efficacy outcomes. Therefore, this paper highlights the need for more multicentral, large sample-size trials with subgroup analysis to determine the best practices for personalised AIS management. ◄

Declarations

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NARRATIVE REVIEW

Your Mouth is the Mirror of Health and Disease of the Gastrointestinal System: Oral Mucosal Manifestations of Gastrointestinal Disease

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Abstract

Introduction: Over the last few years, the importance placed on multi-disciplinary approach to patient care has been increasing. One such relationship is the between a dentist and medical practioner.

Methods: This narrative review examines the manifestations of gastrointestinal diseases in the oral mucosa. Understanding the interconnection between the oral cavity and gastrointestinal conditions can help with early diagnosis and can serve as an indicator of disease progression. The aim of this narrative review is to underscore the importance of recognising these suspicious oral mucosal signs and understand the bi-directional relationship between oral health and systemic well-being.

Results: The findings highlight the need to integrate oral examinations in patients at high risk of or with suspected or current gastrointestinal diseases. A holistic approach will help in better patient care and overall patient outcomes.

Discussion: There exists a gap in diagnostic approaches for oral manifestations of gastrointestinal diseases, which must be bridged to efficiently facilitate early diagnosis and treatment of these diseases. Understanding this interplay between oral health and systemic well-being will significantly improve patient care and outcomes, optimizing resource allocation for healthcare systems and promote disease prevention.

Keywords: Oral mucosa, Gastrointestinal disease, Inflammatory bowel disease, Coeliac disease, Gastro-oesophageal reflux disease, Helicobacter pylori, Hepatobiliary system

Introduction

The World Health Organization recognizes oral health as a "key indicator of overall health, well-being and quality of life"[1]. The oral cavity can be regarded as the mirror of systemic health and a gateway to the gastrointestinal tract. Several systemic diseases may have manifestations in the oral cavity and, vice-versa, oral diseases may affect systemic health[2]. Correlations between alterations in the oral cavity and systemic conditions have been widely reported, particularly gastrointestinal conditions[3]. The oral cavity and gastrointestinal tract share a common embryological origin; therefore, it is unsurprising that gastrointestinal diseases may have oral manifestations[4]. These signs or symptoms could manifest prior to or in the absence of gastrointestinal signs and may persist after the disease has resolved. They may occur alongside other systemic indications and could be a direct result of the disease itself or due to secondary effects such as ineffective nutrient assimilation by a compromised bowel[5]. Oral symptoms may not be sufficient to provide a definite diagnosis, although they may be useful in establishing required investigations and differential diagnoses. They may serve as an early warning sign, prompting a proactive approach to targeted diagnosis and treatment. Clinicians may establish a more informed and nuanced understanding of the patient's overall health, potentially uncovering subtle signs of gastrointestinal issues that might otherwise go unnoticed. For a healthcare system, early detection and intervention would assist in optimizing resource allocation and achieving greater cost-effectiveness. Awareness of these signs and links to gastrointestinal diseases would allow for public health interventions and preventative strategies to mitigate the risk of developing or exacerbating the below mentioned conditions.

A considerable number of gastrointestinal conditions of varied nature including, genetic, inflammatory, infectious, and metastatic may produce alterations in hard oral tissue and oral mucosa. These include inflammatory bowel diseases, coeliac disease, gastro-oesophageal reflux disease, and metastasis[3]. The oral mucosa includes lining mucosa (labial and buccal mucosa, soft palate), masticatory mucosa (gingiva, dorsal tongue surface, hard palate) and specialised mucosa (filiform papillae, fungiform papillae). This essay will discuss the oral mucosal manifestations of several gastrointestinal diseases and syndromes and explain

their histopathology, prevalence, and associations with disease progression.

Inflammatory bowel disease

Inflammatory Bowel diseases (IBD) comprise Crohn's disease and ulcerative colitis. Inflammatory bowel diseases affect the intestinal tract and may have extra-intestinal involvement, such as in the oral cavity. Particularly in IBD, these manifestations may assist in the diagnosis and monitoring of disease progression or exacerbations. Manifestations of oral signs in inflammatory bowel disease show a male predominance and are more common in children[6]. Studies indicate that the oral cavity is a useful source of diagnostic material in paediatric population with suspected IBD, particularly in Crohn's disease. This suggests the need for routine examination of oral cavity and dental health in children and increased awareness of disease-specific manifestations amongst physicians[7]. Additionally, oral lesions are more common in Crohn's disease compared to ulcerative colitis[6]. This is of particular significance since in up to 60% of patients with Crohn's disease oral lesions may be the primary presenting sign[8]. IBD patients, when compared to matched controls, have significantly increased prevalence of periodontitis, caries, and non-specific oral lesions[9]. Patients with ulcerative colitis are 27% and Crohn's patients are 89% more likely to seek more comprehensive and expensive dental treatment compared to controls[9]. A survey suggested only 12.5% of patients remembered being counselled by their physicians on the link between IBD and oral lesions and only 10% received treatment for them[9]. Evidence suggests the higher prevalence of oral health problems and higher need for dental treatment in IBD patients is partly due to the nature of their systemic disease. As such, the need for a multi-disciplinary approach in IBD is essential to ensure optimal dental and oral health in patients and to allow for early diagnosis and treatment, particularly in paediatric population.

Crohn's disease

Crohn's disease is a chronic inflammatory condition characterised by non-caseating granulomas in the gastrointestinal tract, commonly the terminal ileum and colon[6]. The prevalence of oral lesions in Crohn's disease patients is higher with perianal and proximal gastrointestinal tract involvement[6]. They may be the primary presenting signs preceding gastrointestinal symptoms in up to 60% of Crohn's patients[6]. Generally, patients with active Crohn's have been reported to have a higher degree of oral lesions, although the type of lesion has no association with intestinal disease activity. Oral lesions can be specific or non-specific depending on the presence of granulomas on histopathology. Diffuse labial, gingival and mucosal swelling and fissuring are specific signs of Crohn's disease[10]. A distinctive feature that may appear is cheilitis granulomatosa which is a manifestation of orofacial granulomatosis[11]. Particularly in children, this granulomatous inflammation of the lips or buccal mucosa may be indicative of underlying disease. A systematic review determined 40% of children with orofacial granulomatosis had concurrent intestinal Crohn's[12]. On histology, it is characterised by noncaseating giant cell granulomas and epithelioid histiocytes[13]. Given the appropriate clinical background, the microscopic presence of granulomas is considered diagnostic of oral Crohn's disease[17]. In adults, cheilitis granulomatosa can occur as a part of Melkersson-Rosenthal syndrome, a rare presentation of orofacial granulomatosis, which includes facial palsy and fissured tongue[6]. Additionally, a combination of deep, transverse, and longitudinal ulcers separating portions of the mucosa may create a cobblestone appearance in the oral mucosa[10]. This is usually seen in the posterior buccal mucosa and causes pain, especially during speaking or eating[14]. White reticular mucosal tags in the labial and buccal vestibules and retromolar regions are also specific to Crohn's disease[14]. Cobblestoning and mucosal tags are considered pathognomonic for Crohn's disease, although are not necessarily concurrent with intestinal disease activity[10]. Persistent and deep linear ulcerations with hyperplastic margins may also be found in the buccal sulci[10]. Oral Crohn's disease may also manifest with mucoginigivitis, i.e. hyperplastic and granular gingiva[10]. Non-specific oral lesions include aphthous stomatitis and angular cheilitis[10]. Aphthous stomatitis is one of the more prevalent lesions among Crohn's patients reported in up to 27% of patients[10]. The correlation of recurrent aphthous ulcers with disease activity has not been established although during active disease lesions can be more severe[10].

Ulcerative colitis

Ulcerative colitis is characterised by non-granulomatous inflammation limited to the mucosa of the rectum and colon[6]. Pyostomatitis vegetans is an oral lesion highly associated with ulcerative colitis and is a specific marker of active disease or exacerbations[16]. It is most commonly found in the buccal gingiva, labial gingiva, and buccal mucosa. Pyostomatitis vegetans are white-yellow pustules with an erythematous and oedematous mucosal base. The pustules may rupture and coalesce to form snail-track ulcers[10]. It is also seen in Crohn's patients although it is non-specific[10]. Histologic features include intraepithelial and subepithelial microabscesses with eosinophil and neutrophil infiltrates[15]. Hyperkeratosis and acanthosis can be present as well[15]. Pyostomatitis vegetans tends to resolve itself with adequate control of underlying inflammatory bowel disease[16]. Non-specific lesions include aphthous ulceration, angular stomatitis, and superficial haemorrhagic ulcers. Some reports of ulcerative colitis suggest 4.3% of patients presented with non-specific lesions during active flare-ups suggesting a correlation with disease activity[6]. Hairy leukoplakia and halitosis (bad breath) have been associated with the long-term use of corticosteroids and immunosuppressive agents in the treatment of ulcerative colitis and Crohn's disease[15].

Coeliac disease

Coeliac disease or gluten-sensitive enteropathy is an autoimmune condition associated with villous atrophy[10].

Several oral signs and symptoms have been recorded in patients with coeliac disease. Dental enamel defects, specifically enamel hypoplasia and delayed tooth eruption are common oral manifestations[3]. Oral mucosal manifestations such as atrophic glossitis are observed secondary to anaemia and hematinic deficiencies such as iron, B12 and folate[17]. Atrophic glossitis is the atrophy of the filiform papillae of the tongue, causing a smooth, glossy, and red appearance which may be patchy or involve the entire dorsum of the tongue[17]. Active coeliac disease is associated with dysfunction of salivary glands and decreased salivary flow rates, which may present as glossopyrosis and xerostomia post-gluten exposure3. Some studies have associated recurrent aphthous-like ulcers due to hematinic deficiencies with Coeliac disease. Cheng et al. (2010) observed aphthous ulcers were more common in untreated patients with coeliac disease compared to control (42.4% vs. 23.2%)[18]. These may manifest as single or multiple recurrent ulcers with erythematous halo and a yellow or grey floor3. However, there is a lack of consensus in literature supporting the relationship between aphthous ulcers and coeliac disease[18]. Rarely, dermatitis herpetiformis, a dermatological manifestation of coeliac disease may present as oral erythematous-purpuric macules, vesicles, erosions and ulcers, which can affect the alveolar ridge, buccal mucosa and tongue[10]. Additionally, a study examined the detection of anti-endomysial and anti-tissue transglutaminase autoantibodies in cheek biopsies from patients with coeliac disease, suggesting oral mucosal involvement in the disease[19].

Considering that 50% of patients with coeliac disease do not exhibit digestive symptoms at the time of diagnosis, it is essential for physicians to be aware of possible indicating oral signs of coeliac disease[10]. Although, the evidence for cheek biopsies for the diagnosis of coeliac disease is insufficient, oral lesions could still be useful in raising suspicion for asymptomatic coeliac disease.

Gastro-oesophageal reflux disease

Gastro-oesophageal reflux disease involves the regurgitation of gastric contents into the oesophagus due to the weakening of the lower oesophageal sphincter causing recurrent symptoms such as heartburn[20]. Dental erosion is the most common extra-oesophageal manifestation, commonly affecting the lingual or palatal surface of anterior teeth[10]. Damage may vary from loss of enamel to exposure of dentin[10]. Enamel erosion is directly proportional to the contact time with gastric acid[17]. Assessing the extent of enamel loss may allow gastroenterologists to approximate the frequency and duration of the disease.

Oral mucosal lesions may result from direct acid or acidic vapour contact[21]. There is a lack of data establishing the effect of gastro-oesophageal reflux disease on the oral mucosa. However, a case-control study found palatal and uvula mucosal erythema to be a significant clinical sign of gastric-oesophageal reflux disease, manifesting in 21.5% of patients in the study[22]. Additionally, xerostomia, halitosis and oral burning sensations were reported in 54.5%, 49.2% and 43.2% of patients in the study respectively[22]. Furthermore, Silva et.al (2001) found microscopic alterations in the palatal mucosa of gastric-oesophageal reflux disease patients[23]. Upon morphometric analysis of the palatal epithelium, epithelial atrophy and increased fibroblasts were detected. The results of a cross-sectional study indicated that aphthous ulceration, gingivitis and angular cheilitis are correlated with gastric-oesophageal reflux disease and dental erosion[24]. They found 60% of participants to have soft-tissue aphthoid lesions i.e. ulcerative lesions on the buccal mucosa, soft/hard palate mucosa, tongue and uvula[24]. There is no consensus as to whether these lesions are directly related to gastric-oesophageal reflux disease as several studies have failed to find statistically significant differences between symptomatic and control groups[24].

Compared to diseases such as inflammatory bowel disease and coeliac disease, evidence supporting the significance of oral lesions in gastro-oesophageal reflux disease is lacking. However, acknowledging the link between gastro-oesophageal reflux disease and dental erosion is essential as it may be associated with debilitating dentition and complex restorative therapy for patients in the long term[21]. In a survey of gastroenterologists, only 42% strongly agreed that such an association existed in adults[21]. Failure to diagnose early signs of erosion can result in significant damage to dentition and the masticatory system, therefore, promoting awareness of this association has become imperative.

Gastritis, Peptic Ulcer Disease and Helicobacter Pylori

Helicobacter pylori (H. pylori) infection is one of the leading causes of chronic gastritis and peptic ulcer disease, including gastric and duodenal ulcers[25]. Studies have determined that the oral cavity may serve as an extra-gastric reservoir for H.pylori[25]. H.pylori has been isolated from the saliva, tongue, and supra-gingival and subgingival plaque[26]. This can be attributed to the low concentrations of antibiotics that reach oral fluids, dental plaque and periodontal pocket[25]. Recent studies show patients with chronic gastritis have a higher prevalence of H.pylori in the dental plaque than in the stomach, suggesting the role of the oral reservoir as a source of infection and re-infection[27]. Wang et. Al (2014) concluded the successful eradication of gastric H. pylori bears a significant relationship to oral infection from H. pylori[28]. Therefore, poor dentition and oral hygiene may be contributing factors in a patient with H.pylori-associated disease. Antibiotics are the first-line protocol to combat H.pylori associated peptic ulcer disease and gastritis[25]. Even though, gastric eradication is successful, reinfection is still possible due to the poor penetrance of antibiotics in the oral cavity and dental biofilm[25]. Gastroenterologists must consider this aspect of recurrence and highlight the importance of good dental hygiene in patients with a history of H.pylori associated gastric disease.

The oral presence of H.pylori has been associated

with recurrent aphthous stomatitis, glossitis, halitosis, gingivitis and dental caries[25]. Birek et. Al (2007) postulated a relationship between H.pylori and recurrent aphthous ulcers, as 71.9% of recurrent aphthous ulcer samples tested had H.pylori deoxyribonucleic acid (DNA)[29]. They suggested H. pylori may be a cofactor in the pathogenesis of recurrent aphthous ulcers, especially in people sensitised through gastric colonisation and mucosal attachment[30]. H.pylori-associated chronic atrophic gastritis and autoimmune chronic gastritis can lead to iron deficiency anaemia and pernicious anaemia, caused by vitamin B12 malabsorption. In severe cases, this can manifest as atrophic glossitis and persistent aphthous-like ulcers that are responsive to replacement therapy[10]. Peptic ulcer disease may manifest in the oral cavity as an erythematous tongue with a slimy yellowish coating and congestion and dilatation of sublingual veins[10].

Diseases of the hepatobiliary system

Chronic cirrhotic liver disease

Oral manifestations of cirrhotic liver disease include jaundice and prolonged bleeding[25]. Excess bilirubin in the blood results in its accumulation in the oral mucosa[25]. Patients with jaundice have a diffuse, uniform, yellow discolouration of all mucosal surfaces[31]. Bilirubin has a high affinity for elastin, therefore mobile oral tissues with higher elastin particularly the lingual frenum and soft palate are more severely affected[17]. Examination of these regions may provide useful diagnostic clues in patients with darker skin or physiologic conjunctival pigmentation and to clinically assess the extent of jaundice[17]. Due to thrombocytopenia and deficiency in coagulation factors, additional oral manifestations may include petechiae on the palate and gingival bleeding[32]. Spider angioma or spider naevus is a vascular lesion caused by abnormal dilation of central arterioles with radiating thin-walled vessels[33]. They are characteristic of chronic liver disease with a specificity of 95% and may manifest in the mucosa of the oral cavity[33]. Patients with severe liver disease, as classified by B/C on Child-Pugh scale, are more frequently prone to oral candidiasis, due to overuse of antibiotics and immunosuppressants[34]. In paediatric populations with hyperbilirubinemia, biliverdin may deposit in teeth during calcification causing a permanent pigmentation of teeth[34]. Studies have suggested that the prevalence of oral lesions such as angular cheilitis, strawberry-looking lips with erosions, smooth and atrophic tongue, and petechiae are statistically significant in children with liver diseases[35]. Some cases of congenital hepatic disease such as biliary atresia have presented with pigmentation of the gingiva, gingivitis and green-staining of the teeth[36].

Hepatitis C infection

One of the leading causes of chronic liver disease is Hepatitis C (HCV) infection. There is some evidence of a relationship between HCV and oral lichen planus. Oral lichen planus is a T-cell-mediated chronic inflammatory disease of the oral mucosa, characterised by white papules and plaques with a reticulated appearance[37]. The evidence varies by region, wherein epidemiological data suggests that lichen planus may be significantly associated with HCV infection, mainly in Southern Europe and Japan[38]. Oral lichen planus typically has a bilateral distribution and most commonly appears on the buccal mucosa, tongue, gingiva, and possibly labial mucosa and lower lip[39]. Studies determined that patients with lichen planus are reported to have a 5 times higher risk for HCV seropositivity[31]. The mechanism is assumed to involve cell-mediated cytotoxicity induced by HCV[31]. The prevalence of general liver disease in patients presenting with lichen planus ranges between 0.1 to 35% with the erosive variant of lichen planus being predominant[40].

Metastatic disease

Metastasis of primary tumours to the oral and maxillofacial region is rare, representing only 1% of all oral cancer[41]. Oral cavity metastasis typically involves the mandible, with only 16% affecting the oral soft tissues, most commonly the gingiva and tongue[42]. Although rare, several cases involving colorectal adenocarcinoma metastasis in the oral cavity have been observed in the mandible and oral mucosa. In a literature review conducted by Lanca et al. (2023), in 22% of patients, the metastasis to the oral cavity was identified before the primary colorectal adenocarcinoma[43]. Mucosal manifestations reported include gingival overgrowths and tongue mass[44]. Gingival metastases resemble a hyperplastic or reactive lesion, such as pyogenic granuloma, peripheral giant cell granuloma, or fibrous epulis[43]. Other soft tissue manifestations are submucosal masses and ulceration. Lesions on the gingiva have been associated with chronic inflammation that attracts malignant cells. Malignant cells may be entrapped by the rich capillary network of chronically inflamed gingiva. Oral pain and mandible and maxillary masses were reported in hard tissue metastasis[45,46]. It has been hypothesised that metastasis to the mandible and maxilla involves the Baston Plexus[43]. Increased pressure in the abdomen can lead to haematogenous dissemination of malignancy in the vertebral venous plexus, affecting the oral hard tissue[43]. According to Maria et al. (2021), cases of gastrointestinal stromal tumour metastasis to the buccal soft tissues and mandible have also been reported[47]. The malignant form of oral acanthosis nigricans has been reported as a rare marker of intestinal malignancy, especially gastric adenocarcinoma[48]. These lesions present as hyperpigmented areas and are characterised by papillomatosis of the lips, palate, gingiva, and tongue[49]. Histologic features of oral acanthosis nigricans include true acanthosis, epithelial papillary hyperplasia and hyperkeratosis[50]. Distant metastasis of primary gastrointestinal tumours to oral mucosal and hard tissue is extremely rare and is considered a poor prognostic sign.

Peutz-Jeghers syndrome

Peutz-Jeghers syndrome is an autosomal dominant genetic disorder characterised by benign hamartomatous polyps in the gastrointestinal tract, mainly the small

intestine[17]. Another characteristic manifestation is oral mucosal melanotic macules[17]. Intraorally, the lesions are usually flat, painless, brown-pigmented patches of the buccal mucosa, tongue, or labial mucosa[17]. These are found in 95% of patients with Peutz-Jeghers syndrome and usually become pronounced in childhood[51]. It often precedes gastrointestinal symptoms and is an essential clinical sign of the disorder. Pigmentation may fade after puberty but often persists in the buccal mucosa[52]. Upon histology, prominent basal layer melanin hyperpigmentation is observed without an increase in melanocytes[17]. Mucocutaneous pigmentation is a key diagnostic factor for Peutz-Jeghers syndrome. Unlike other gastrointestinal conditions, the oral-mucosal manifestation is a part of the disease diagnostic criteria, whereas other manifestations as in inflammatory bowel disease are suggestive or prognostic as in metastatic disease.

Plummer-Vinson Syndrome

Plummer-Vinson syndrome is a rare disorder characterised by the triad - microcytic hypochromic anaemia, dysphagia, and oesophageal webs. The most common oral mucosal manifestation is atrophic glossitis[53]. It serves as a suggestive factor of disease and should promote further testing into suspected Plummer-Vinson Syndrome. Others include angular cheilitis, burning mouth syndrome, erythematous mucositis, oral candidiasis, pale oral mucosa and recurrent aphthous stomatitis[53]. These oral manifestations can be attributed to iron deficiency anaemia. Plummer-Vinson syndrome predisposes a patient to develop squamous cell carcinoma of the upper gastrointestinal tract, especially the pharynx and proximal oesophagus[54]. A typical clinical picture would be that of a middle-aged woman with weight loss, symptoms of anaemia, oesophageal webs on endoscopy, glossitis, angular cheilitis and koilonychia[54].

Conclusion

Our mouth is the mirror of systemic health and disease of the gastrointestinal system. The presence or extent of oral manifestations allows for monitoring of disease severity and prognosis. The response of oral tissue may also reflect the success of the management of gastrointestinal disease. Although the frequency of oral manifestations is variable and non-specific, these alterations may precede the underlying disease and therefore can facilitate an opportune diagnosis and encourage vigilance. For some disease, these oral lesions may serve as a key diagnostic factor, aiding in raising suspicion and further testing.

In patients with suspicious oral alterations such as those discussed in this essay and suspected gastrointestinal disease, a multidisciplinary approach of general practitioners, dieticians, gastroenterologists, dentists are essential to improve patient care, disease management and outcome. Integrating a clinical examination of the whole oral cavity into a gastrointestinal examination can promote early diagnosis. The importance of oral hygiene and lifestyle modifications such as tobacco and smoking cessation is essential in these cases to promote gastrointestinal and systemic well-being[15]. For a healthcare system, integrating this step in a gastrointestinal examination, may in the long run optimise resource allocation and cost-effectiveness, by promoting early diagnosis and treatment. By encouraging further research into the oral signs of these diseases, public health interventions and prevention strategies may also be successfully put into place. ◄

Declarations

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LITERATURE REVIEW

Current Antiviral Options for Therapeutic Management Of SARS-CoV-2 Infection

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Abstract

Introduction: The COVID-19 pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has led to an urgent need for effective antiviral treatments. This paper provides an overview of current antiviral options for therapeutic management of SARS-CoV-2 infection, focusing on their mechanism of action, clinical efficacy and considerations for specific populations.

Methods: A literature review of several antiviral drugs have been evaluated for their effectiveness. Notable options include ritonavir-boosted nirmatrelvir, remdesivir, molnupiravir, favipiravir, and chloroquine/hydroxychloroquine.

Results: Ritonavir-boosted nirmatrelvir has shown promising results in reducing the risk of hospitalisation or death when administered within 5 days of symptom onset. Remdesivir has demonstrated efficacy in reducing hospitalisation rates and improving clinical outcomes in certain patient populations. Molnupiravir, has shown a reduction in rate of hospitalisation, although caution is advised regarding its use in pregnancy. Favipiravir and chloroquine/hydroxychloroquine have shown varied efficacy and are not currently recommended by organisational guidelines. Considerations for special patient populations, such as pregnant individuals, are discussed. While antiviral therapies may offer potential benefits, the evidence for their use in pregnant individuals is limited, emphasising the need for a case-by-case multidisciplinary approach.

Discussion: While antiviral treatments play a crucial role in managing SARS-CoV-2 infection, more research is needed to fully understand their efficacy and safety profiles, particularly in specific patient populations. Vaccination remains the most effective method for preventing severe COVID-19 presentations. .

Keywords: COVID-19, SARS-CoV-2, antivirals

Introduction

The COVID-19 outbreak was declared a pandemic on March 11th 2020[1]. The rapidly transmitting and deadly severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had increased the number of cases by 13-fold in the preceding two weeks1. To date, there have been over 774 million cases, and 7 million deaths, with new cases continuing to be reported[2, 3]. The most common symptoms of COVID-19 include fever, dry cough, dyspnoea, chest pain, fatigue, and myalgia[4].The less commonly reported symptoms like diarrhoea, nausea, vomiting, headache and dizziness[4]. The majority of infections are asymptomatic or with mild symptoms.

However, people with one or multiple health conditions are at a higher risk for severe COVID-19. A metanalysis found that hospitalised patients with comorbidity have a 20.3% chance of requiring intensive care, putting a huge strain on the health care system at the peaks of the pandemic[5].

Treatment of COVID-19

The evidence for antivirals in treatment of COVID-19 is severely limited[6]. Trials were carried out in unvaccinated individuals and prior to the new variant of concern, Omicron. The strongest evidence for treatment of COVID-19 is: venous thromboembolism prophylaxis, corticosteroids and in rapidly deteriorating patients, the use of tocilizumab[6]. In addition, it is recommended that anti-virals only be used in patients with high risk for severe disease[6]. No one treatment is favoured over the other, but the decision is made on the basis of clinical context, such as the patient's renal function and medication interactions[6].

COVID-19 Viral Life Cycle

Rapid assessment began of current antiviral drugs that may be effective in treating COVID-19. A virus can attach to a host cell, penetrate it and once inside the cell, carry out further steps (uncoating, reverse transcription, transcription, translation) of replication before being released and infecting other host cells[7]. Antivirals target the virus lifecycle and can prevent the virus from entering the host cell, replicating, packaging and releasing. There are over 80 antivirals available; the majority used to treat HIV infections and the others for influenzae A and B, cytomegalovirus, hepatitis A and C, and herpes simplex virus[7]. The SARS-CoV-2 lifecycle starts with the attachment to the host cell via co-receptor binding and membrane fusion involving the S spike protein, the host cell's angiotensin-converting enzyme 2 (ACE2) receptor,

and the cell surface serine protease TMPRSS2[7, 8]. This allows the virus to be enter the host cell by endocytosis or by plasma membrane fusion. The S spike protein has also been found to neutralise antibodies, making it easier for the virus to bind to the host receptors[9]. Viral fusion inhibitors can be used to inhibit this step[7].

Once in the cytoplasm, the virus uncoats and the SARS-CoV-2 RNA is released. Host ribosomes translate the RNA open reading frames (ORF) ORF1a and ORF1b into polyprotein pp1a and pp1b[8, 10]. These polyproteins help in hijacking the host ribosome, which is necessary for viral replication. The polyproteins undergo cleavage by proteases, such as main protease (MPRO), yielding non-structural proteins needed for viral replication and transcription[8, 10]. Thus, inhibiting proteases is a potential strategy to treat SARS-CoV-2[7].

The exact replication of SARS-CoV-2 is not completely understood, but it can be explained using the SARS-CoV model[7]. The non-structural protein (nsp12) forms an RNA-dependent RNA polymerase (RdRp) producing a negative-sense RNA strand, complimentary to the positive-RNA strand template. The negative-sense strand is used to synthesise new positive-sense RNA strands11. It is at this step that reverse transcriptase inhibitors can be used[7].

After replication, post-translational modifications occur in the endoplasmic reticulum-Golgi apparatus compartment. Assembly and budding of the enveloped virus occur in the endoplasmic reticulum before passing through the Golgi apparatus. Here, the mature virus is released in vesicles that leave the cells to infect other cells[7].

Anti-viral Drugs

Ritonavir-boosted nirmatrelvir (Paxlovid)

Ritonavir-boosted nirmatrelvir (Paxlovid) is a protease inhibitor against the protease MPRO[12, 13]. It is orally available and has been shown to be effective against all coronaviruses that infect humans[12]. Ritonavir is a CYP450 3A4 inhibitor and is therefore packaged with nirmatrelvir to increase the latter's concentration to therapeutic ranges. All of patients' medications should be reviewed prior to prescribing Paxlovid due to its CYP450 inhibition effect[6, 12, 13]. For example, it may reduce the efficacy of combined oral contraceptives; therefore, the patient should be advised to use an alternative method of contraception. It is recommended by the Health Service Executive (HSE) that Paxlovid be used in non-hospitalised or hospitalised patients[6]. They must be at least 18 years of age, have no oxygen requirement, have an oxygen saturation greater than 94% and a respiration rate less than 20 breaths per minute[14]. It should be started within five days of symptom onset in a patient with a confirmed COVID-19 polymerase chain reaction test and continued for five days[13, 14].

In the EPIC-HR trial, it was found that Paxlovid reduced the risk of hospitalisation or death by 88% compared to a placebo group[14, 15]. Its high efficacy, paired with it being the only oral antiviral available for treatment of COVID-19, poses a strong argument for Paxlovid to be considered in patients with a high risk of significant medication interactions, and initiates a discussion as to whether the interacting medications can be adjusted or substituted to safer alternatives. Furthermore, it is important to note that the EPIC-HR trial excluded pregnant and lactating individuals, despite ritonavir being used considerably in pregnant individuals with HIV[14, 15]. According HSE guidelines, Paxlovid can be used in pregnant individuals on a case-by-case-basis, if the benefits outweigh the risks[6].

Other considerations are those patients with renal impairment. The normal dose is 300mg of nirmatrelvir and 100mg of ritonavir taken together every 12 hours for five days[6]. For those with an eGFR <60mL/min, the dose should be reduced by half, 150mg of Nirmatrelvir and 50mg of Ritonavir every 12 hours for five days. In those with an eGFR <30mL/min, Paxlovid is not recommended[6].

Remdesivir

Remdesivir is a nucleotide reverse transcriptase inhibitor; the nucleotide analogue inhibits RdRp in the majority of single stranded RNA viruses, such as the coronaviruses[7]. A three-day intravenous. course of treatment is recommended for unvaccinated patients with risk of progressing to severe COVID-19 or the immunocompromised regardless of vaccination status[6]. The patients must also be presenting within eight days of symptom onset, have a confirmed COVID-19 PCR, be over 18 years old and not requiring oxygen therapy[6]. The PINETREE trial[16] showed that a 3-day treatment of intravenous remdesivir resulted in an 87% reduction in risk of hospitalisation or death compared to the placebo[11].

A five-day course of remdesivir may be advised in patients that are on oxygen therapy, and meeting all other inclusion criteria, however, there is limited evidence of efficacy[6]. It is recommended that a loading dose of 200mg be administered on day 1, followed by 100mg on day 2 and 3[6, 11]. Remdesivir is not recommended for treatment of children under 12 years of age, nor in patients with an eGFR <30mL/min[6]. It is also contraindicated in patients with alanine aminotransferase greater than five times the upper limit of normal. as clinical trials observed elevated transaminases with remdesivir treatment[6].

Molnupiravir

Molnupiravir is a ribonucleoside. When incorporated into the host DNA, it induces lethal mutations[11]. 800mg of molnupiravir orally twice daily for five days is recommended by the National Institute of Health, for those greater than 18 years of age but only for when Paxlovid and Remdesivir are not available or clinically appropriate[11].

The MOVe-OUT study found that molnupiravir reduced the rate of hospitalisation by 30%. The trial consisted of randomising 1433 participants, of which 716 received the drug, and the remainder receiving the placebo[17]. The participants were within five days of symptoms onset, unvaccinated with lab-confirmed COVID-19, with mild-to-moderate symptoms, and at least one risk factor for severe COVID-19[17].

Molnupiravir is not recommended for use in pregnancy due to the foetal toxicity reported in animal

studies[11]. If other therapies are not available than molnupiravir can be used if the patient is beyond the 10 weeks of gestation. However, it is imperative that the patient is fully informed of the risks, and advised of the option to participate in the surveillance programme[11]. For individuals that are lactating, there is limited evidence that molnupiravir may cause adverse effects in infants[11]. It is recommended by the FDA that lactating people stop breast feeding while undergoing treatment until 4 days after their last dose[11].

Favipiravir

Favipiravir is a guanine nucleotide analogue and acts as an RdRp inhibitor[7]. In its activated phosphoribosylated form, favipiravir-RTP, it inhibits RdRp and arrests RNA synthesis. It was initially developed for influenza; and was approved in Japan in 2014 for resistant influenza infection with several other countries following suit[18]. Due to its wide spectrum of activity, favipiravir is a candidate for COVID-19 treatment. There is limited and varying evidence on its efficacy, which has deterred said drug from being recommended by organisational guidelines[19]. A systematic review and meta-analysis of clinical trials of favipiravir for treatment of COVID-19 concluded that the antiviral is associated with significant clinical improvement in most patients. They also concluded that the treatment conferred no serious side effects but also had no significant effect on mortality[20]. The study also noted limitations to the clinical trials, such as varying doses and duration of treatment, small sample sizes, and efficacy due to patient multi-drug pharmacy[20]. In all, the mixed results and low power trial data are indicative that more clinical trials with larger sample sizes need to be carried out[20].

Chloroquine/Hydroxychloroquine

Chloroquine and hydroxychloroquine are used to treat malaria as well as autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis. They have shown to increase the endosomal pH and inhibit SARS-CoV-2 cell fusion with the host cell membrane[21]. Chloroquine also inhibits the glycosylation of the ACE2 receptor, which may interfere with the fusion of SARS-CoV-2[22]. Chloroquine/hydroxychloroquine have been shown to have immunomodulatory effects hence their use in autoimmune diseases, and it is hypothesised that this may potentiate the effects of COVID-19 infection as well. Although these two medications demonstrate antiviral activity, neither hydroxychloroquine nor hydroxychloroquine plus azithromycin, demonstrated a reduction in the viral load of the upper or lower respiratory tract in non-human primates[11, 23].

In the UK RECOVERY trial, 1561 patients were randomly assigned to receive hydroxychloroquine[24]. In the hydroxychloroquine group, they were less likely (59.6%) to be discharged from the hospital alive within 28 days compared to the usual-care group (62.9%). Furthermore, patients in the hydroxychloroquine group that were not undergoing mechanical ventilation at baseline had a higher frequency of invasive mechanical ventilation or death[24]. In the WHO Solidarity trial, an international randomised controlled trial, hydroxychloroquine was given to hospitalised patients and withdrawn from patients due to its low efficacy; there was no difference in mortality when compared to the control group[25]. In a systematic review and meta-analysis of 51 studies (n = 61,221; nine randomised controlled trials; 42 observational studies), there was no significant reduction in mortality, length of hospital stay, time to fever resolution, incidence of mechanical ventilation or time to a negative SARS-CoV-2 PCR test with treatment with hydroxychloroquine with or without azithromycin[26]. Thus, the NIH and the HSE do not recommend chloroquine/hydroxychloroquine alone or in combination with azithromycin for the treatment of SARS-CoV-2[6].

Pregnant individuals

While most pregnant individuals infected with COVID-19 present asymptomatically or with mild-to-moderate disease, there is data suggesting that SARS-CoV-2 infection in pregnant individuals may be at increased risk of severe disease, as well as higher rates of mortality, morbidity, ICU admissions and need for ventilation[6]. Vaccinations remain the most effective method in preventing severe COVID-19 disease in pregnant individuals[6]. They present similarly to non-pregnant individuals and thus, management is the same as per national guidance. With pharmacological treatment, the potential risks of COVID-19 infection should out-weigh the unknown risk of the drug on the pregnant individual or foetus.

The evidence of antivirals for treatment of COVID-19 is very limited. There is currently no evidence for use of nirmatrelvir in pregnancy but that the patients should be reviewed on a case-by-case basis[6]. In nonclinical reproductive toxicity studies, intravenous remdesivir did not show any adverse effects on embryofoetal development in pregnant rats and rabbits, at four times the recommended human dose[27]. Furthermore, an RCT of remdesivir in treatment of EBOLA included six pregnant women with no adverse effects on the pregnancy reported[28]. With that being said, there is still limited evidence on antivirals for treatment of COVID-19 in pregnant individuals, and thus a case-by-case multidisciplinary approach is necessary[6].

Conclusion

Whilst individuals infected with SARS-CoV-2 are mostly asymptomatic, it can cause serious disease and mortality[6]. Thus, antiviral therapies are being investigated for the treatment of SARS-CoV-2. Antivirals target various steps and enzymes of viral replication like fusion, proteases, RNA-dependent RNA polymerases, and reverse transcriptase. Viral replication may be particularly active early in the infection course; thus, antiviral therapy may have the best effect before the illness progresses to the inflammatory late stage[6].

The HSE recommends Paxlovid, and remdesivir for treatment of COVID-19 in the early stages of infection. Paxlovid is administered orally for five days within five days of symptom onset and after medication review

for interactions. Remdesivir, an intravenous drug, should be given for three days and started within seven days of symptom onset in patients with a confirmed COVID-19 PCR test. For those requiring supplemental oxygen therapy, a five-day course of remdesivir may be considered, using a multidisciplinary approach[6].

The NIH also recommends the use of Paxlovid and remdesivir, but additionally recommends molnupiravir, if the former two are unavailable or clinically inappropriate11. Molnupiravir should be given per oral twice daily for 5 days. However, it should be cautioned when used in pregnancy because limited evidence demonstrated foetal toxicity in animal studies[11].

There are also drugs that have yet to be approved by organisations but that have the potential to treat COVID-19. For example, favipiravir is a candidate but there is limited and varying evidence of its efficacy[7]. Thus, more research is needed into the use of favipiravir for the treatment of SARS-CoV-2.

Both the HSE and the NIH recommend against the use of hydroxychloroquine with or without azithromycin[6,11]. A systematic review found that papers reported no significant reduction in mortality, length of hospital stay, time to fever resolve, incidence of mechanical ventilation, and time to a negative SARS-CoV-2 PCR test[26]. Furthermore, the UK RECOVERY trial found that the hydroxychloroquine patient group were less likely (59.6%) to be discharged from the hospital alive within 28 days, compared to the usual-care group (62.9%)[24].

As per HSE guidelines, treatment of pregnant individuals infected with COVID-19 should receive the same treatment is non-pregnant individuals. There is still limited evidence on antivirals for treatment of COVID-19 in pregnant individuals, and thus a case-by-case, multidisciplinary approach is necessary[6].

The strongest evidence for treatment of COVID-19 is venous thromboembolism prophylaxis, corticosteroids and in rapidly deteriorating patients, the use of tocilizumab[6]. The best prevention of severe infection remains to be vaccination. Currently, the HSE only recommends the antivirals Paxlovid and remdesivir for early treatment in those unvaccinated or immunocompromised with risk of severe disease progression and not requiring supplemental oxygen[6]. More research into the efficacy of antivirals for treatment of COVID-19 is needed, including special patient groups such as pregnant and lactating individuals. ◄

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Declarations

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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LITERATURE REVIEW

A Literature Review of the Analytical Toxicology of Fentanyl Derivatives

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Abstract

Introduction: Fentanyl is a synthetic opioid with the potential to cause life-threatening adverse effects in overdose scenarios, including sedation and respiratory depression. The rising prevalence of fentanyl-related substances since the mid-2000s constitutes a serious concern.

Methods: This literature review offers a comprehensive overview of the current information available concerning fentanyl derivative toxicology, including testing methodologies in biological fluids, metabolism, pharmacokinetics and levels found in overdose or deaths associated with their use.

Results: There are significant knowledge gaps in the current literature on fentanyl derivatives, partly due to their extremely low serum concentration. Lower limit of detection figures are typically in the range of 0-1 ng/mL, necessitating the use of highly sensitive testing methodologies. Immunoassays are widely available but limited in their ability to distinguish between derivatives. Gas chromatography-mass spectrometry offers untargeted data acquisition and vast mass spectral libraries; however, this technique has lengthy preparation times and limited sensitivity. Liquid chromatography-mass spectrometry has recently been used with quadrupole time-of-flight or orbitrap technology to offer tentative identification of compounds without library searching. However, the real weapon needed to tackle the ongoing fentanyl crisis is a technique which can assist in the prediction of unknown compounds.

Discussion: The recent advent of a machine learning model applicable to mass spectra offers promising potential to predict the structure and spectra of previously unknown fentanyl analogues. Moreover, increased funding is required to enhance the sensitivity of current fentanyl detection techniques in combating the overdose epidemic.

Keywords: fentanyl, fentanyl derivative, analytical toxicology

Introduction

History and Therapeutic Indications of Fentanyl

Fentanyl was first synthesised by Dr. Paul Janssen in 1960 and was approved for medical use in the USA in 1968[1]. Fentanyl and its analogues have therapeutic uses in analgesia and anaesthesia, especially for cancer and chronic pain patients who experience "breakthrough pain" or develop a tolerance to other opioids[2]. Therapeutic analogues used for human analgesia and anaesthesia include alfentanil, remifentanil and sufentanil. Misuse of fentanyl within the US and Europe has been documented since the 1970s, originally mixed with heroin[3]. The longest documented fentanyl epidemic occurred in Estonia, escalating after the outlaw of opium poppy growth in Afghanistan, originally the world's largest opium poppy supplier[4]. Fentanyl abuse has been particularly on the rise for the past two decades, with an increasing number of deaths related to the abuse of fentanyl-derived opioids. A 2012 report by the European Union Drugs Agency (EMCDDA) suggested the high-risk nature of fentanyls would dissuade opioid users and that the fentanyl crisis may have "built-in breaks" in some respects[5]. However, the number of deaths involving fentanyl and other synthetic opioids is increasing, with 73,838 deaths reported in the US in 2022[6]. Fentanyl analogues are being created at a faster rate than they can be scheduled (i.e. categorised based on abuse potential). In the US, this has led to temporary scheduling orders being placed on fentanyl-related substances in 2018, which has been extended multiple times[7].

Chemistry

Fentanyl is the prototype of the 4-anilidopiperidine class of synthetic opioid analgesics. Its molecular formula is $C_{22}H_{28}N_2O$, and its molecular weight is 336.471 g/mol. Its synthetic name is N-(1-(2-phenethyl)-4 piperidinyl-N-phenyl-propanamide)[8]. Various non-pharmaceutical fentanyl (NPF) derivatives have since been developed by adding various substituents to the basic molecule, enhancing its analgesic potency to 10,000 times that of morphine. Examples of such changes include the replacement of the piperidine ring for pyrrolidine and the replacement of the phenyl group in the phenethyl-part of the molecule for some aromatic heterocycles,

mainly for thiophene and tetrazole[9].

Mechanism of Action

Fentanyl is a selective agonist of mu-opioid receptors. Its rapid onset, duration of action, potency and risk of overdose are attributable to its significant lipid solubility[10]. Mu-opioid receptors are G-protein coupled receptors (GPCRs), comprising a single polypeptide chain with 7 transmembrane domain receptors that interact with heterotrimeric g-proteins[11]. Mu (μ) receptors are involved in the neuromodulation of nociception, respiration, gastrointestinal activity as well as stress, temperature, memory, motivation and endocrine function. Agonism of Mu receptors by fentanyl is responsible for its clinical use in analgesia and anaesthesia but is also responsible for adverse effects experienced by patients, such as opioid-induced constipation, drowsiness and respiratory depression[12].

Fentanyl also causes muscle rigidity in the chest wall via dopaminergic pathways, decreasing respiratory rate and the efficacy of cardiopulmonary resuscitation (CPR)[13]. High or multiple doses of Naloxone, an opioid antagonist, may be required for reversal.

Forensic Toxicology

Forensic toxicology is primarily carried out to determine the role fentanyl plays in drug-related deaths and criminal cases. Low concentrations of fentanyl in postmortem samples often lead to difficulties in detection and interpretation[14]. The minimum effective concentration (MEC) for fentanyl analgesia is approximately between 0.6 - 1.5 ng/mL, while the MEC for anaesthesia is between 10 - 20 ng/mL. The lethal dose for fentanyl is 2mg, however, for a synthetic opioid such as carfentanil, a lethal dose can be just 0.0002mg. This great variation in potency precludes the straightforward detection, identification and cross-comparison of derivatives. These illicitly synthesised fentanyl can also be mixed with other substances such as cocaine, heroin, and ecstasy, amplifying the risk of drug overdose and death, often without the user's knowledge[15].

Aim

The aim of this literature review is to perform a thorough toxicological analysis of fentanyl derivatives, primarily in the legal context, exploring biological testing methods, metabolism, pharmacokinetics and levels found in overdose and deaths associated with their use.

Methodology

Meta-analyses, literature reviews, systematic reviews and case reports on fentanyl and their derivatives were sought from various search engines and websites, including CAS SciFinder, PubMed and Google Scholar. Keywords searched included: fentanyl, fentanyl derivatives, fentanyl analogues, metabolism, fentanyl metabolites, forensic, toxicology, analysis, pharmacokinetics, urine, blood, plasma, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). The 'search by structure' feature on Chemical Abstracts Service (CAS) SciFinder enabled access to further papers relevant to specific fentanyl analogues. Studies were excluded if they were deemed irrelevant: eligibility assessment was performed by the independent reviewers and disagreements were resolved by consensus.

Results

Adverse effects: Acute and Chronic

Depending on the route of administration, fentanyl has an onset of action of minutes to hours. Fentanyl overdose is a medical emergency. Acute overdose symptoms include constricted pupils, clammy and cold skin, discoloured or pale skin, nausea and vomiting, choking sounds, slurring or loss of speech, sedation and respiratory depression. Severe overdose can lead to respiratory arrest, cardiac arrest, or a severe anaphylactic reaction, resulting in sudden death. Long-term fentanyl use can result in chronic adverse effects, including opioid tolerance, dependence and addiction[12]. Fentanyl tolerance leads to increased dosage requirements to elicit the desired effects, which increases the risk of overdose.

Prevalence: Statistics

Due to a powerful potency and reduced half-life, fentanyl and its analogues account for many overdoses worldwide. The full extent of the synthetic opioid crisis is presumed to be underreported due to a lack of routine diagnostic monitoring. Drug overdose deaths involving opioids continue to rise, with 80,411 deaths in the U.S. in 2021[6]. Fentanyl has comprised the majority of all drug overdose deaths in the U.S. since 2018, overtaking heroin[6].

Metabolism

As seen in Table 1 , the metabolism routes of fentanyl analogues vary depending on their chemical structure. Fentanyl has various sites for metabolic transformation. It consists of a heterocyclic tertiary aliphatic amine containing two different phenyl rings and an aromatic amide function. Tertiary aliphatic amines are bio transformed through a reversible reaction into tertiary amine oxides. In addition, the tertiary amines undergo N-dealkylation through carbinolamine. When this process occurs on the phenylethyl side chain, a phenylacetaldehyde is also produced, which immediately oxidises into phenylacetic acid. Oxidation at the 2-position of the piperidine ring leads to the production of a carbinolamine. This subsequently transforms into a more stable aminoaldehyde, resulting in ring cleavage. Aromatic rings undergo oxidation, producing the equivalent phenolic derivatives. Moreover, benzylic positions are more prone to oxidation. Amide functions usually undergo hydrolysis, and oxidation of the carbon chain is also frequent.

In humans, fentanyl is principally metabolised in the liver by CYP3A4 into norfentanyl. This occurs through oxidative N-dealkylation at the piperidine ring by hepatic CYP3A4 and 3A5 isoenzymes: the main pathway of metabolism. The inactive metabolites and under 10% of the intact molecule are primarily excreted in urine and faeces. Less than 1% is metabolised by alkyl hydroxylation, combined N-dealkylation and hydroxylation, or amide

Table 1. **Analytical Toxicology Data for Fentanyl Derivatives**

Compound	Method	Parent drug/ Metabolites	LLOD	Conc. found	Patient condition	Other	Reference
Acetylfentalnyl	ELISA GC-MS/MS LC-MS/MS	Acetylfentanyl mainly undergoes N-dealkylation to acetyl norfentanyl	0.1-1.0 ng/g	Case 1: In heart whole blood: 155 ng/g In urine: 126 ng/g Case 2: In urine: 570 ng/g	Case 1: death Case 2: survived	Both individuals self-administered mepirapram and acetylfentanyl; case 1 administered intravenously, case 2 administered by inhalation	34, 35
Acrylfentanyl	LC-MS (urine)	Acrylfentanyl metabolites: Nor-acrylfentanyl Hydroxyacrylfentanyl Dihydroxycacrylfentanyl Hydroxymethoxyacrylfentanyl	0.05ng/ mL	Case 1 – 0.3ng/mL Case 2 – 0.95ng/mL Case 3 = 0.32ng/mL	All cases death	Case 3 – 0.95ng/mL furanylfentanyl detected	36
Benzoylfentanyl	NMR LC-HRMS ELISA	BZF metabolites: norBZF, despropionoylfentanyl and a hydroxylated-BZF pFBF metabolites: norpFBF, parafluorofentanyl and a hydroxylated-pFBF					37, 38
Benzoylbenzylfentanyl	LC-QTOF-HR MS	10 Metabolites detected (B1-10) B1-$C_{18}H_{24}N_2O_2$: Amide hydrolysis + benzyl dihydrodiol formation B2-$C_{25}H_{28}N_2O_3$: Benzoyl dihydrodiol formation B3-$C_{18}H_{22}N_2O$: Amide hydrolysis+ benzyl hydroxylation B_4-$C_{18}H_{20}N_2O$: N-dealkylation B5-$C_{25}H_{28}N_2O_3$: Benzyl dihydrodiol formation B6-$C_{18}H_{22}N_2$: Amide hydrolysis B7-$C_{25}H_{26}N_2O_2$: Benzyl hydroxylation B8-$C_{18}H_{22}N_2O$: Amide hydrolysis + N-oxide formation B9-$C_{26}H_{28}N_2O_3$: Benzyl dihydroxylation + methylation B10-$C_{25}H_{26}N_2O_2$: N-oxide formation Most abundant = Normetabolite (B4)					39
Benzodioxole-fentanyl	MS						40
Acetylbenzylfentanyl	LC-QTOF-HR MS	7 metabolites detected (A1-7) A1-$C_{20}H_{26}N_2O_3$: N-Phenyl Dihydrodiol formation A2-$C_{13}H_{18}N_2O$: N-dealkylation A3-$C_{20}H_{26}N_2O_3$: Benzyl Dihydrodiol formation A4-$C_{26}H_{32}N_2O_8$: Hydroxylation + Glucuronidation A5-$C_{20}H_{24}N_2O_2$: Hydroxylation A6-$C_{20}H_{24}N_2O_2$: Benzyl Hydroxylation A7-$C_{20}H_{24}N_2O_2$: N-oxide formation Most abundant = Normetabolite (A2)					39

Table 1. **Analytical Toxicology Data for Fentanyl Derivatives (Cont.)**

Compound	Method	Parent drug/ Metabolites	LLOD	Conc. found	Patient condition	Other	Reference
Butyrfentanyl	LC-MS/MS LC-QTOF	Butyrfentanyl was metabolised to carboxyfentanyl, hydroxyfentanyl, norfentanyl and desbutyrfentanyl Most abundant = Carboxyfentnayl and hydroxyfentanyl	LLOQ:1 ng/mL	In heart blood (9 hours after death): 39 ng/mL In urine (at autopsy): 1100 ng/mL	Death		41
Carfentanil	GC-FID GC-MS LC-MS/MS (blood and urine)	Norcarfentanil		4.2 µg/L .0042 /mL 4.2ng/mL (Blood)	Death		42
Cyclopropylfentanyl	GC-MS HPLC-DAD (Blood/ urine)	Cyclopropyl-norfentanyl, N-methyl cyclopropylnorfentanyl	0.5ng/mL	20.4ng/mL (Blood)	Death		43
Cyclopentylfentanyl	LC-MS-MS	N-dealkylation, mainly alkyl hydroxy metabolites, 4-ANPP (amide hydrolysis), N-oxide and ketone formation	<0.5 ng/mL	0.5-1000 ng/mL (Blood)			24, 44, 45
Methoxyfentanyl	LC-MS (blood)	O-demethylation → O-demethyl- Hydroxylation on ethyl linker → HO-ethyl- (can be precursor → Deethylphenyl-) or phenyl → HO-phenylAbove metabolites combined → HO-HO-ethylphenyl-and HO-ethylphenyl-O-demethyl Cleavage of amide bond → deamide- Hydroxylation on aniline phenyl → Deamide-HO-phenyl Hydroxylation on ethylphenyl linker → Deamide-HO-ethylphenyl O-glucuronidation O-demethyl → O-demthyl-glucoronide		0.022- 0.056 mg/kg (blood)	Death		46
Methoxyacetylfentanyl	LC-MS-MS	Demethylmethoxyacetylfenta nyl (O-demethylation), 4-ANPP (amide hydrolysis), normethoxyacetylfentanyl (N-dealkylation), alkyl/ aryl hydroxy metabolites and phase II conjugates		Mean concentrations for 11 cases quantitatively confirmed was 17.7 ng/ mL		Estimated relative potency to fentanyl: 0.3	24, 44
2,2,3,3-Tetramethyl-cyclopropylfentanyl (hydrochloride)	MS	Monohydroxylations and dihydroxylations and subsequent further oxidation steps					47

Table 1. **Analytical Toxicology Data for Fentanyl Derivatives (Cont.)**

Compound	Method	Parent drug/ Metabolites	LLOD	Conc. found	Patient condition	Other	Reference
Tetrahydrofuranyl fentanyl (THF-F)	GC-MS HPLC-TOF FTIR-ATR GC-(MS)-IR condensed phase	Nortetrahydrofuranylfentanyl (N-dealkylation), alkyl/aryl hydroxy metabolites, ring opening of the tetrahydrofuranyl ring and 4-ANPP (amide hydrolysis); (N-dealkylation is the proposed predominant metabolic step; (hydroxylation of the piperidine ring and the phenylethyl side chain, N-oxidation and amide hydrolysis to 4-ANPP were also observed)			THFF has caused death in at least 15 drug-using individuals to date	Estimated relative potency to fentanyl: approximately 0.2 Highly selective for the mu-opioid receptor Closely related to furanylfentanyl (Differs by bearing a fully saturated furanyl ring, instead of an unsaturated ring)	16, 48, 49
3-phenylpropanoylfentanyl	NMR GC-MS FTIR	Mainly metabolised through N-dealkylation to form nor-metabolites					50
2-fluorofentanyl (ortho-fluorofentanyl)	LC-QTOF-MS		≤0.5ng/ mL	Case 1: Hospitalised. 2.5ng/mL (Serum) Case 2: Autopsy post-mortem: 2.4ng/mL (blood) 3.9ng/mL (urine)	Case 1: Male in early 20s, lost consciousn ess and respiratory function, CPR and Naloxone administer ed. Discharged . Found dead at home a few days later. Case 2: Case 1: Male in early 20s, lost consciousn ess and respiratory function, CPR and Naloxone administer ed. Discharged from hospital.	Case 1: While hospitalised. Additional toxicological findings: (Serum) Ethanol: 1.0 g/L Alprazolam 96 ng/mL Benzoylecgonine: (not quantified) Amphetamine: (not quantified) Paracetamol: (not quantified) Case 2: Autopsy. Additional toxicological findings: Blood Alprazolam: 16 ng/mL Desmethyldiazepam: 3.8 ng/mL (most likely metabolite of previously ingested diazepam) 7-Aminoclonazepam: 34 ng/mL(inactive metabolite of Clonazepam, may be formed post mortem) THC: 21 ng/mL GHB: 22 µg/mL (Urine) Ethanol THCA GHB Concluded that death was caused by ortho-fluorofentanyl.	51`

Table 1. **Analytical Toxicology Data for Fentanyl Derivatives (Cont.)**

Compound	Method	Parent drug/ Metabolites	LLOD	Conc. found	Patient condition	Other	Reference
4-fluoro-isobutyrylfentanyl (4F-iBF)	Paper 1: LC-HRMS HPLC-TOF GC-MS-IR Paper 2: MS, LC-MS/MS	Paper 2: solvent = chloroform-d Metabolites: Isobutyrylfentanyl hydrochloride, nor-4-fluoro-isobutyrylfentan yl (nor-4F-iBF) hydrochloride, nor-4-chloro-isobutyrylfentan yl (nor-4Cl-iBF) hydrochloride, and nor-isobutyrylfentanyl (nor-iBF) hydrochloride				GC-MS analysis of 4F-iBF and 4F-BF (4-fluoro-butyrfentanyl) display very similar mass spectrometry fragmentation patterns. Analytical reference standards or access to reference spectra required for both substances to distinguish between them. ELISA may not distinguish between 4F-iBF and fentanyl due to structural similarity	52, 53
4-chloro-isobutyrfentanyl (4Cl-iBF)				Serum: 5-45 ng/mL Urine: 11-136 µg/mmol creatinine		Acrylfentanyl, 4F-iBF, THF-F	54
2-Furanylfentanyl (2-Fu-F)	LC–MS-MS		LLOQ: 0.100 ng/mL	Blood concentration ranges of 0.15-0.30 ng/mL. Case 3: levels of 2-Fu-F: 8.7 ng/mL (heart blood), 5.5 ng/mL (femoral blood) Approx 30 ng/mL (vitreous humor)	Case 3: Death.	Research and testing completed in the context of cases of DUID (Driving Under the Influence of Drugs) and in post-mortem cases. Case 3: Cause of death was combined effects of heroin, fentanyl, diphenhydramine and 2-Fu-F. Accidental death.	55
Valerylfentanyl	MS-MS GC-MS LC-MS/MS	Paper 2: 4-Anilino-N-phenethylpiperidi ne (4-ANPP) Hydroxylated valerylfentanyl Valeryl norfentanyl	0.05 ng/mL LLOQ: 0.10 ng/mL	0.10 - 21 ng/mL (post-mortem blood)	4 selected cases, all deceased	Not detected as the sole intoxicant in these cases. Other opiates, cocaine, benzodiazepines and ethanol were most commonly detected.	56, 57
3-Methylcrotonylfentanyl (3-MCF) C24H30N2O	UHPLC-QTO F-MS	6 Metabolites detected (A1-A6) A1-C24 H30N2O2: Hydroxylation A2-C16H22N2O: N-dealkylation A3-C24H28N2O3: Carboxylation A4- C24H28N2O3: Carboxylation A5-C24H28N2O2: Carbonylation A6- C24H30N2O3: Dihydroxylation Abundance: A1>A3>A5>A2>A6>A4 and A3> A6> A4> A1> A2> A5 after 30 and 240 min					58

Blank data indicates no available data as of 18/04/2023

hydrolysis to the inactive compounds hydroxyfentanyl, hydroxynorfentanyl, and despropionylfentanyl[16].

Drug-drug Interactions

Due to its metabolism, fentanyl should not be combined with CYP3A4 inhibitors, including macrolide antibiotics, azole-antifungal agents, or protease inhibitors. This inhibition will decrease fentanyl's degradation to inactive norfentanyl. CYP3A4 inducers such as carbamazepine and phenytoin will increase fentanyl clearance, reducing its effect. Fentanyl increases serotonin levels, so combination with any MAO inhibitors, SSRIs or any drug that increases serotonin levels can cause serotonin toxicity[17]. Fentanyl can also reduce the clearance of sedative drugs such as midazolam[18].

Analytical Confirmation and Methodologies Used: Concentrations in Biofluids

Biofluids typically used in fentanyl analysis include blood, urine and saliva. Prior to analysis, biofluid samples are prepared/purified by either solid-phase extraction (SPE) or liquid-liquid extraction (LLE) methods. Methodologies used for analysis depend on the type of biofluid for analysis, the sensitivity and selectivity of the analytical method, and the required detection limit.

As seen in Table 1, the lower limit of detection (LLOD) ranges for fentanyl derivatives are typically extremely low (0-1 ng/mL), meaning analytical methods to determine fentanyls in biofluids must have a high sensitivity. Immunoassays are antibody-based methods commonly used to screen samples for fentanyl. These methods include lateral flow assays (LFAs), heterogeneous immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), and homogeneous immunoassays, such as enzyme multiplied immunoassay technique (EMIT)[19]. Despite their ubiquity in fentanyl toxicology labs, immunoassays are limited in their utility. Standard immunoassays are unable to detect new opioids, are limited to a set number of drugs, have limited cross-reactivity, and cannot distinguish between derivatives of fentanyl. Specific assays can be utilised, but often are not used routinely, and require confirmation with specific chromatographic techniques[20]. LFAs are the fastest and least expensive option, however, they are not as sensitive as other immunoassays. Wang et. al developed a high throughput homogeneous enzyme immunoassay (HEIA) that can detect fentanyl in urine at a cut-off concentration of 2 ng/ml, offering much greater sensitivity than LFAs[21].

As mentioned previously, specific ELISAs can also be used to detect fentanyls, however this process is limited by its manual nature. ELISA testing additionally requires confirmation by mass spectrometry (MS), a technique which is often not available in hospital laboratories[19]. GC-MS offers the ability to obtain untargeted data, which can be searched in vast mass spectral data libraries to identify compounds in biological samples. However, sensitivity remains an issue. Typically, detection values range between 1-10 ng/ml is not sufficient for detection of the more potent fentanyl analogues, which are often found in incredibly low concentrations[22]. Additionally, GC-MS methods are not able to directly analyse non-volatile, polar or thermally labile drugs, necessitating the use of lengthy sample preparation techniques and thus limiting the application of GC-MS to routine rapid testing.

Liquid chromatography with tandem mass spectrometry (LC-MS/MS), on the other hand, has high sensitivity and offers relatively rapid detection. LC-MS/MS can detect fentanyl in plasma samples with a lower limit of qualification (LLOQ) of 0.05ng/ml and norfentanyl at 0.25ng/ml[23]. Fogarty et al. developed a technique using LC-MS/MS that has allowed the pharmacological effects of methoxyacetylfentanyl and cyclopropylfentanyl to be associated with quantitative values in samples, a breakthrough for two of the more elusive fentanyl derivatives[24]. This achievement illustrates the potential for LC-MS/MS to offer insights into other derivatives. However, currently, a universal library for LC-MS/MS does not exist, as does for GC-MS, and many forensic laboratories do not have MS/MS capabilities. Additionally, LC-MS/MS is often targeted, it will only detect substances for which the method is specifically designed. It is also time-consuming; results are rarely produced in sufficient time to contribute to the real-time care of patients or detection of outbreaks. Overall, this technique is useful in the profiling of illicit fentanyl compounds, however, it is not capable of solely conquering the ongoing threat of synthetic opioid creation.

Liquid chromatography electron ionisation mass spectrometry (LC-EI-MS) is a promising testing alternative. LC-MS offers the advantage of injection at room temperature (circumventing the thermal degradation obstacle) of compounds dissolved in aqueous solution, however there is no universal library available for LC-MS/MS. GC-MS offers an extensive library searching capability, which can be employed to full advantage with the myriad fragmentations produced by EI. Put simply, LC-EI-MS combines the advantages of these two techniques, overcoming the limitations of using either in isolation. This technique is rapid and highly sensitive, with the ability to determine fentanyl in plasma between 0.02–10ng/ml and to determine fentanyl and norfentanyl in urine in the range 0.1–50 and 0.102–153 ng/ml, respectively[25].

Other modern testing methodologies have been developed for the detection of fentanyl analogues in various settings, both for forensic toxicology laboratories and for use on the field. Thermal desorption direct analysis in real-time mass spectrometry (TD-DART-MS) is a fentanyl detection technique with potential applications in mobile laboratories, emergency vehicles and hospitals. This approach may be more effective than current ELISA screening and GC/MS analysis techniques, as it offers greater sensitivity. Ion mobility spectrometry (IMS) is another technique offering greater sensitivity than current colourimetric techniques.This approach can also be used to detect fentanyl even in the presence of heroin, making it particularly advantageous for use on

the field[26].

Legislation and Education

Regulation. Fentanyl and its analogues are subject to both international treaties and the laws of individual countries. Fentanyl has been internationally controlled under the 1961 Single Convention since 1964. In the United States, the Drug Enforcement Administration (DEA) schedules drugs, including fentanyl, under the Controlled Substances Act of 1970 and the Controlled Substances Analogue Enforcement Act of 1986[27]. On February 6, 2018, a proactive class-wide scheduling of fentanyl-related substances was initiated, leading to a dramatic fall in fentanyl analogues in the marketplace. The United States Congress has temporarily extended this scheduling, which is to expire in December of 2024. The imminent expiration date has led to renewed pressure for a class-based scheduling strategy and increased research into fentanyl-related substances[27].

Impact on Society. Given the reduced cost, increased potency, more straightforward synthesis and lack of agricultural requirements of fentanyl in comparison to heroin, illicit drug distributors have recently demonstrated increased preference of synthetic opioids over non-synthetic opioids. The price of fentanyl has been reported to be 1% that of heroin in some cases, substantially decreasing expenses. Opium poppy growers will most likely be made obsolete by the rise of synthetic opioids. Farm prices in Mexico have decreased between 60-80%, and Afghanistan's massive opium poppy economy is at risk of the same demise[28].

Opioid users may convert from heroin to fentanyl due to its high potency, increased availability and lower cost[29]. Given that fentanyl has a duration of action approximately 1/2-1/3 that of heroin, synthetic opioids must be administered more frequently to avoid withdrawals, bearing an increased risk of blood-borne illnesses and overdoses[30]. A cross-sectional risk behaviour survey in Estonia found that 62% of participants who injected fentanyl as their primary drug had contracted Human Immunodeficiency Virus (HIV). The survey also demonstrated increased likelihood to share needles, reuse needles and use discarded needles for intravenous injection31. In general, the literature illustrates that fentanyl, and its analogues may become more prevalent than heroin due to increased accessibility for intravenous drug users.

Education and Awareness. Prior to 2017, the highest overdose death mortality rates in Europe were held by Estonia for over a decade. Fentanyl has been a large contributor to this epidemic. Estonia's declining drug overdose mortality rates have been due to many factors, including the distribution of take-home naloxone kits, needle exchange programmes, free and confidential HIV screening and antiretroviral treatment (ART) for HIV positive users, and the closedown of a large producer & distributor of illicit fentanyl. The "Break the Cycle" initiative was introduced in Tallinn, and due to its success was introduced to New York. This programme was developed as a motivational-interview-based initiative. The programme aims to discourage experienced people who inject drugs (PWIDs) from showing other drug users how to inject drugs for the first time, as almost all IV drug users require assistance from experienced PWIDs for their first injection. These programmes and tools have been invaluable in tackling Estonia's opioid epidemic, and their implementation should be considered in other countries[4].

Existing Gaps in our Knowledge. An exhaustive review of all relevant information concerning fentanyl derivatives was precluded by lack of research, and occasionally lack of access to existing literature, on some of the more elusive analogues. Choice of testing methodology and medium for fentanyl detection varied greatly in the articles reviewed, making a comparison of fentanyl levels difficult. More sensitive technology is needed to investigate LLOD values for all fentanyl analogues and to associate their pharmacological effects with levels found in samples.

Discussion

As illustrated by Table 1, fentanyl is often extensively metabolised through demethylation, hydroxylation, N-dealkylation, and amide hydrolysis to form a variety of metabolites. Fentanyl metabolism is primarily mediated by CYP3A4, although other cytochrome P450 (CYP) isoenzymes may make minor contributions32. The main site of metabolism is the liver. The primary metabolite of fentanyl is the norfentanyl form, a nontoxic and inactive piperidine N-dealkylated compound. Other minor metabolites (less than 1%) identified include despropionylfentanyl, hydroxyfentanyl, and hydroxynorfetanyl. All fentanyl metabolites have negligible pharmacological activity.

Our findings have illustrated that typical detection concentrations for fentanyl derivatives are in the low nanogram range, necessitating highly sensitive detection techniques. Fentanyls and their derivatives can be detected using immunoassays, although this technology is limited by inability to detect novel opioids or distinguish between fentanyl derivatives. LFAs are the fastest and least expensive option, however, they are not as sensitive as other immunoassays. The high throughput homogeneous enzyme immunoassay (HEIA) developed by Wang et al. in comparison, offers much greater sensitivity[21].

Certain fentanyl derivatives, such as methoxyacetylfentanyl and cyclopropylfentanyl (Table 1), are present in such low concentrations that they lie outside the scope of routine drug testing. These analogues can only be detected by extremely sensitive techniques, such as that developed by Fogarty et al. using LC-tandem mass spectrometry. This technique has allowed the pharmacological effects found in case reports to be associated with quantitative values found in postmortem specimens, enabling much more effective investigation into methoxyacetylfentanyl and cyclopropylfentanyl overdose[24]. This new technique, similar to the HEIA devised by Wang et al., illustrates how current detection technologies can be enhanced to

yield more data on potent fentanyl derivatives. Further research is needed to develop similar techniques for the analysis of other elusive fentanyl analogues, such as benzodioxolefentanyl and 3-fluorofentanyl, which remain largely uninvestigated, as seen in Table 1.

LC-EI-MS is another promising testing alternative, combining the advantages of LC-MS with EI and GC-MS to offer a rapid and highly sensitive detection technique. However, the real challenge of combating the ongoing fentanyl crisis lies in the detection and prediction of unknown compounds. LC high resolution mass spectrometry (LC-HRMS) quadrupole time-of-flight or orbitrap technology offers several benefits in the detection of fentanyl, including tentative identification of compounds without library searching, and the untargeted acquisition of data that can be applied to analysis of new synthetic substances and the elucidation of their metabolic pathways. However, this technology is not readily available in most clinical laboratories.

Machine learning models are among the most promising new techniques in the prediction or classification of unknown samples as fentanyl analogues, alongside their current role of enhancing the accuracy of overdose analysis. Machine learning involves extracting patterns from mass spectra of known fentanyl analogues to assist in the prediction of unknown fentanyl derivatives[38]. Traditional detection of fentanyl via library matching is greatly enhanced through such models, and machine learning random forest models offer even more significant improvement. Such models have recently been applied to fentanyl analogue detection using sensing technologies such as infrared spectra and surface-enhanced Raman spectra. However, Koshute et al. have been the first to apply machine learning to general fentanyl analogue detection using mass spectra, a more prominent technology in forensic toxicology laboratory analyses[33]. This study has illustrated that random forest models can achieve 99% probability of detection (PD) and 1% probability of false alarm (PFA) against unseen spectra, offering a hugely significant improvement over standard detection techniques such as library matching. Further validation of the model developed by Koshute et al. would be greatly beneficial, potentially through the evaluation of earlier models upon analogues that have been assigned a later date of emergence. Further effort is also needed to adapt the approach to time series of mass spectra, a highly prominent technique in forensic toxicology. These enhancements have the potential to dramatically improve the accuracy of fentanyl detection analysis and are well worth investigating.

Conclusion

We reviewed the current literature available as of April 2023 on fentanyl derivative toxicology. Our findings indicate that fentanyl analogues are currently most often detected in urine and blood, as well as hair and saliva. Fentanyl is extremely potent, minute quantities can elicit powerful effects. This property often leads to challenges in fentanyl detection, as many analogues are present in concentrations that fall outside the range of routine testing. Highly sensitive testing methodologies are needed to perform accurate analogue analysis. The high throughput HEIA developed by Wang et al. offers much higher sensitivity than standard LFAs used for fentanyl detection. However, immunoassay techniques in general are severely limited in sensitivity. LC-MS is a far more sensitive option and has recently been used in conjunction with electrospray ionisation to further enhance fentanyl detection in forensic toxicology laboratories. Other techniques such as TD-DART-MS and IMS are more suited to use on the field in comparison to LC-MS.

The "designer" aspect of fentanyl and its analogues presents another challenge. Testing is necessitated for myriad structures, some of which may be novel, unknown, or yet to be reported in the literature. Machine learning has the potential to provide extremely useful insight into such analogues. The machine learning models described by Koshute et al. have potential application in the prediction of structure and spectra of fentanyl analogues that have not yet been observed but could potentially be synthesised. This enhancement of their model would be highly valuable in combating possible future threats. However, further work is needed on the reliable prediction of mass spectra from chemical structures before this can be achieved. ◄

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Declarations

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. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Key for Analytical Techniques

LC	Liquid Chromatography
GC	Gas Chromatography
MS	Mass Spectometry
LC-MS-MS	Liquid Chromatography Tandem Mass Spectrometry
LC-HRMS	Liquid Chromatography with High-Resolution Mass Spectrometry
LC-QTOF-HRMS	Liquid Chromatography Quadrupole Time of Flight with High-Resolution Mass Spectrometry
UHPLC-QTOF-MS	Ultra-High Performance Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry
HPLC-TOF	High Performance Liquid Chromatography Time-Of-Flight
HPLC-DAD	High Performance Liquid Chromatography with Diode-Array Detection
GC-MS	Gas Chromatography – Mass Spectrometry
GC-MS-IR	Gas Chromatography - Mass Spectrometry - Infrared Spectroscopy
GC-FID	Gas Chromatography - Flame Ionization Detection
FTIR	Fourier Transform Infrared Spectroscopy
FTIR-ATR	Fourier Transform Infrared Spectroscopy Attenuated Total Reflectance
NMR	Nuclear Magnetic Resonance
ELISA	Enzyme-Linked Immunosorbent Assay
LOD	Limit of Detection
LOQ	Limit of Quantification
B	Blood
U	Urine
S	Saliva

LITERATURE REVIEW

An Evaluation of Novel Immunological Biomarkers for the Diagnosis of Neonatal Sepsis in the Emergency Department

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Abstract

Introduction: Sepsis, a common presentation to the Emergency Department (ED), is characterised by a dysregulated and rapidly progressive immunological response causing multi-organ dysfunction. Neonatal sepsis (NS) occurs in infants less than 28 days old and is one of the most common causes of paediatric death. The current gold standard for diagnosis is blood culture. However, a novel combinational approach looking at levels of biomarkers which elevate upon the onset of bacterial sepsis is recommended for diagnosis and prognosis.

Methods: A literature review on biomarkers of NS in the ED.

Results: MiRNAs exhibit altered patterns in NS, and due to their specificity and ease of detection, they also make for potential high yield markers in the ED.

Discussion: While new biomarkers hold promise, further studies are needed before standardisation and recommendation for clinical practice.

Keywords: Neonatal sepsis, Early-onset sepsis, Biomarkers, Late-onset sepsis, Presepsin, Procalcitonin, microRNA

Introduction

Sepsis is a severe medical condition characterised by a dysregulated immunological host response to infection causing multi-organ organ dysfunction[1]. Sepsis is a common presentation to the emergency department (ED) which can rapidly progress if left untreated. Neonatal sepsis (NS) is defined as sepsis presenting in infants less than 28 days old[2]. NS can be divided into early-onset sepsis (EOS), within the first 72 hours of life, and late-onset sepsis (LOS), which is between the first 72 hours and 28 days of life[2]. Every year, one million deaths occur from NS, with an overall mortality rate of 17.6%[3]. The rapid onset and progression of NS necessitates a timely and accurate diagnosis to enable pathogen-specific treatment. However, current diagnostic methods of NS have a high analytical time period and a low positive predictive value (PPV), which hinder an accurate and timely diagnosis of NS. As such, this paper presents a discussion into the current microbiological diagnostic methods and new promising biomarkers in the evaluation of NS. As EOS is usually diagnosed in the postnatal ward prior to discharge, this paper primarily focuses on LOS as it more commonly presents to the ED due to community-acquired infections.

Epidemiology and Risk Factors

Neonatal sepsis remains one of the most common causes of paediatric mortality and disability worldwide[4,5]. Research indicates that high-income countries present the lowest case fatality rates (CFRs), while the highest CFRs are found in low-income countries[6]. Risk factors associated with NS, particularly LOS, include immaturity, intravascular catheters, mechanical ventilation, prolonged parenteral nutrition, surgery, hospitalisation and underlying cardiovascular or respiratory diseases[7,8].

Pathophysiology and Presentation

Recognition by pattern recognition receptors (PRRs) is the initial step of the immune response in sepsis; the release of inflammatory and regulatory cytokines cause endothelial cell activation, increased adhesion, and localised recruitment of immune cells[10].

In neonates, soluble cytokine/receptor antagonists modulate inflammatory activity[10]. Presepsin is a soluble fragment of CD14, a receptor found on the surface of immune cells which has elevated levels in NS. Similarly, procalcitonin, a precursor molecule to the hormone calcitonin, rises a few hours after bacterial infection. Lastly, microRNAs may regulate gene expression to modulate immune homeostasis in response to NS.

The clinical manifestations of NS can be highly non-specific and encompass a range of symptoms such as fever, respiratory distress, lethargy or irritability, seizures, protruding fontanelles, aversion to feeding, jaundice, haemorrhage, abdominal swelling, and dysregulation of body temperature[10].

Common Causative Agents of Late-Onset Neonatal Sepsis

Compared to EOS, causative agents of LOS are typically acquired from the surrounding environment where transmission is primarily via the mother to their child[2]. The most common causative agent of LOS is coagulase-negative staphylococcal species (CoNS), culpable for more than 50% of LOS in high-income countries[2]. It is important to note that the common causative agents of LOS differ internationally; CoNS is less implicated in LOS in developing countries[10].

In terms of management, neonates promptly receive intravenous (IV) antibiotics, once clinical suspicion for sepsis arises[10,11]. Supportive therapy including cardiopulmonary support, IV nutrition, and blood product transfusion can also be utilised[13].

Diagnosis of Late-onset Neonatal Sepsis

As soon as LOS is suspected, blood samples should be taken via venepuncture, preferably at two peripheral sites, and cultured for both aerobic and anaerobic microbes[13]. Prompt treatment with empiric antibiotics with coverage for nosocomial infection (vancomycin plus an aminoglycoside are recommended) should be started even before confirmation with laboratory data[2].

The gold standard for confirmation of LOS has predominantly been a positive blood culture or polymerase chain reaction (PCR). However, these conventional microbiology laboratory techniques take a considerable amount of time, with blood cultures having a turnaround rate of between 48 and 72 hours[14]. Furthermore, CoNS-positive blood cultures should be interpreted with caution, as CoNS is part of the skin flora and is often a common contaminant[10]. The National Institute of Child Health and Human Development (NICHD) Neonatal Research Network published that in order to confirm a culture-proven diagnosis of CoNS sepsis, two positive blood cultures or one positive blood culture alongside elevated C-reactive protein (CRP) are required, due to the high likelihood of contamination in blood cultures[10].

Recent studies have also emerged that question the sensitivity of blood cultures in proving LOS infections. In a study conducted on post-mortem haematological findings of newborns, pre-mortem blood cultures were negative in 14% of newborns with confirmed infection at autopsy[15]. Additionally, another study reported that only 8.9% of 164,744 blood cultures obtained from very low birth weight (VLBW) infants with clinically suspected LOS were positive[15].

Wynn suggests that when diagnosing LOS there may be a significant risk of false negatives in blood culture[15]. Potential reasons for decreased sensitivity of blood cultures include low blood volume drawn, improper timing of blood collection, sub-optimal number of samples collected, prenatal antibiotic use, and limited laboratory capabilities[14]. Zea-Vera and Ochoa report that culture negative cases represent the majority of NS cases in developing countries[10]. The possible decreased sensitivity of blood cultures due to limited laboratory or hospital resources should be taken into consideration when interpreting negative blood cultures, especially in developing countries. Furthermore, regular optimal blood draws (≥1ml) may be hard to achieve in very low birth weight (VLBW) infants and may increase the need for blood transfusions, further adding to the limitations of blood cultures14. In addition, 68% of septic infants present with low level bacteremia (≤10 colony forming units (CFU)/ml), with 42% of septic infants yielding ≤1 CFU/ml. This only further increases the likelihood of a false negative result, with a study showing that low level bacteremia coupled with suboptimal blood draws (0.5ml) can yield false negative results in up to 60% of cases[10]. This may suggest that a negative blood culture alone, especially when conducted under suboptimal conditions, might be inadequate to rule out LOS. Additionally, the ratio of culture-confirmed to culture-negative sepsis cases in term-infants admitted to neonatal units in Norway was 91:1447 (1:16)[16], further questioning the reliability of a negative blood culture for ruling out LOS.

Other biomarkers commonly used in aiding the diagnosis of sepsis include C-reactive protein (CRP) and elevated immature to total (I:T) neutrophil ratio[2]. However, these biomarkers have a low PPV, and thus are typically used as "rule-out" tests[2,14,15]. CRP typically increases at six to eight hours after the onset of sepsis and peaks at 24 hours[2]. Continuously normal levels of CRP are indicative of an absence of bacterial LOS[2], and potentially could be used in determining the discontinuance of empirical antibiotics in neonates[14]. Higher I:T neutrophil ratios are also associated with progressively increasing odds of positive LOS infection[17]. Two normal I:T ratios along with a sterile blood culture have a maximum NPV of 100%, thus making I:T ratio laboratory investigations a useful tool in ruling out LOS[14]. However, the presence of elevated I:T ratios alone is not confirmatory for LOS; elevated I:T ratios are found in up to half of uninfected infants, and have a poor PPV of 25%[2].

The historical gold standard of LOS diagnosis, combined with the low PPV of current biomarkers indicate the need for further research into new diagnostic biomarkers with higher PPVs and faster turnaround rates, which would expedite reliable NS diagnoses in the ED.

Biomarkers

Procalcitonin

Procalcitonin (PCT) has emerged as a promising biomarker for sepsis. PCT is synthesised as a prohormone of calcitonin by thyroid C-cells and is an acute phase protein. Several tissues secrete PCT in response to diverse stimuli, including cytokines (IL-6, IL-1β and TNF-α) and

lipopolysaccharide (LPS). PCT acts as a chemoattractant for blood monocytes[18]. In healthy neonates, PCT levels increase until postnatal days 2–4. In contrast to CRP, PCT does not increase with local bacterial infections, non-infectious inflammatory reactions and viral infections. This is due to the ability of IFN-γ, commonly produced in viral infections, to downregulate PCT. PCT is a potential biomarker to discriminate between bacterial and viral aetiologies of infection, though this has yet to be conclusively established in neonates.

PCT levels rise rapidly 2-4 hours after an endotoxin challenge and peak within 6-8 hours, thus making it more sensitive than CRP as a biomarker for the early diagnosis of NS[19]. Along with a turnaround time of less than 1-2 hours (with assays performed on site), this rapid rise makes PCT a potentially useful biomarker for the early diagnosis of NS in the ED, allowing for timely therapeutic measures to be initiated whilst reducing sepsis-related mortalities[20,21]. Moreover, the increase in serum PCT appears to correlate with the severity of both disease and mortality. PCT levels also offer prognostic value, as response to antibiotic treatment leads to a rapid decrease in PCT levels, showing potential for use in PCT guided antibiotic therapy.[20]

More recent reviews have focused on comparing PCT with CRP. Eschborn et al. identified 39 studies directly comparing the two. They established that the mean sensitivity of PCT for LOS was 88.9%, compared to 77.4% for CRP. Specificity values were 75.6% for PCT and 81.7% for CRP respectively[22]. Therefore, PCT was found to be slightly more sensitive and specific in NS diagnosis. Furthermore, PCT appeared less affected by other factors such as mode of delivery or surgical procedures. This was confirmed by a 2021 systematic review which concluded that PCT showed better sensitivity, specificity, PPV and NPV than CRP, making it a sensitive, independent and useful biomarker for the early diagnosis of NS[23].

A 2023 meta-analysis highlighted the importance of biomarkers in enhancing diagnostic capability for NS in low- and middle-income countries, where disease burden is high and standard diagnostic modalities are potentially lacking[24]. A total of 23,179 neonates were included in this study, which assessed CRP, ESR, WBC and PCT. While both PCT and CRP displayed good discriminatory value for NS, their specificity alone was insufficient for a definitive diagnosis of sepsis.

Determining normal cut-off values for PCT is critical due to its physiological increase after birth. A meta-analysis identified a PCT cutoff of 2-2.5ng/ml as showing the highest sensitivity and moderate accuracy for neonates with suspected sepsis. While this cut-off has a high sensitivity, its specificity is low. The need to maintain a low false-negative ratio due to the high mortality rate of NS makes this acceptable[25]. Importantly, this meta-analysis suggests that the use of two different cut-offs could improve accuracy with higher cut-offs for neonates with EOS than those with LOS.

PCT's implication in bacterial NS makes it a promising diagnostic biomarker with prognostic value and the potential for PCT guided antibiotic therapy. These factors can help to shorten the NS diagnosis time, and reduce mortality rates in the ED.

Presepsin

Presepsin (sCD14-ST) is the truncated form of sCD14 (soluble cluster-of-differentiation 14), a glycoprotein associated with the membrane surface of various immune cells, such as monocytes, neutrophils and macrophages. CD14 acts as a high-affinity receptor for LPS complexes[26]. sCD14 is the soluble form of the receptor, and it is found in the plasma, where it is cleaved by circulating plasma proteases[27,28,29]. Upon bacterial infection, the CD14-LPS-LBP (lipoprotein binding protein) complex activates a TLR4-specific pro-inflammatory signalling cascade which results in the release of cytokines. The complex is then internalised into a phagolysosome, where cathepsin D cleaves its N-terminal. This results in the formation of presepsin, which is released into the bloodstream[26].

Several studies have shown that presepsin is a reliable biomarker for NS, as it is unaffected by confounding perinatal inflammatory factors. Clinical studies have demonstrated that the plasma levels of presepsin tend to increase significantly at onset of sepsis and septic shock. Compared to other biomarkers, presepsin seems to have a better sensitivity and specificity, a crucial feature for the diagnosis and treatment of NS[27,30].

In order to use presepsin as a first-line biomarker for NS, it is crucial to have clear reference values. In 2015, Pugni et al. conducted a hallmark study aimed at identifying reference ranges of presepsin in neonates. Upon collection of 100 µL of whole blood from each neonate, they were able to establish a reference for both term (466.5-791 pg/mL) and preterm neonates (503-864 pg/mL)[31]. These values are important for comparison with presepsin serum levels in EOS and LOS. Researchers have compared the two and concluded that initial presepsin levels in both EOS and LOS were significantly higher than in healthy neonates[32, 33].

Several studies have proven the diagnostic and prognostic reliability of presepsin, along with additional advantageous properties. For example, the concentration of presepsin increases very early in bacterial infection, and the measurement can be taken directly in the ED within 17 minutes[34]. Compared to the time-consuming microbiological isolation of blood cells, presepsin offers a faster, yet sensitive and specific opportunity to diagnose sepsis. Additional studies have also demonstrated its high pooled sensitivity (92%) and pooled specificity (86%), in regards to all NS cases[35].

A systematic review aimed to compare the diagnostic accuracy of PCT alone, CRP alone, PCT combined with CRP andand presepsin alone in the diagnosis of NS. It found that the pooled sensitivity of CRP was the weakest, and the pooled negative likelihood ratio (NLR) of PCT + CRP, as well as presepsin alone, were less than CRP alone. The area under the curve (AUC) for presepsin (was 99%,) was greater than PCT + CRP (96%), PCT (91%) and CRP (85%), highlighting that the combination of PCT and CRP or presepsin alone improves can improve the accuracy of diagnosis of NS[19]. This suggests that a combination of multiple biomarkers as well as clinical findings is best for decision making during the diagnostic process.

Furthermore, past studies on presepsin levels on arrival in the ED have shown that it can be useful for risk stratification. In fact, presepsin plasma levels within 24 hours upon arrival were noticeably lower among NS survivors. This correlation can be a useful therapeutic marker, as presepsin plasma levels tend to decline by the 7th day of treatment with an effective antibiotic therapy. Complications and increased mortality are associated with persistently high presepsin plasma levels[36]. Such a relationship can be attributed to the prominent role of CD14 during infection. It is also worth noting that these observations have primarily been made on adult patients[36]. Hence, it is necessary to test this hypothesis further on neonates.

One caveat applies to the use of presepsin as a diagnostic biomarker in NS. High concentrations of this molecule in the blood can also be a consequence of other clinical conditions, particularly renal failure[37]. There are other clinical conditions which can interfere with normal presepsin range values, such as translocation of the intestinal microbiome, but these have yet to be studied in neonates[37].

Interleukin-6

Interleukin-6 (IL-6) is another potentially useful biomarker for NS. IL-6 production increases immediately after exposure to bacterial endotoxins, and is primarily released by monocytes, endothelial cells and fibroblasts. Its concentration rises rapidly with the onset of the inflammatory response, but has a short half-life and normalises within 24 hours[38]. IL-6 has a pro-inflammatory effect, inducing CRP and PCT release, as well as T-cell differentiation and B-cell maturation. IL-6 has been found to be elevated in neonates with both EOS and LOS[39].

A meta-analysis found encouraging results for IL-6 as a diagnostic biomarker for NS. The pooled sensitivity and specificity were 79% and 84% respectively, with an area under the ROC curve (AUC) of 89%. This shows favourable accuracy of IL-6 for predicting NS, reinforced by the results of the meta regression analysis confirming that the diagnostic accuracy of IL-6 remains unaffected by confounding variables such as cut-off levels of IL-6 assay or birth weight[40]. Another meta-analysis found similar results[39]. IL-6 demonstrated a specificity of 88% and a sensitivity of 82%. In addition, the area under the summary receiver operating characteristics curve AUC was notably high at 92%[39]. This statistical technique can be used in meta-analyses of diagnostic tests, and the closer the value is to 1, the more accurate a diagnostic test is. This suggests that IL-6 holds promise as an accurate tool in diagnosing NS.

Ye et al. conducted a study evaluating numerous cytokines, including IL-6, as biomarkers compared to CRP. IL-6 levels (>12.5 pg/mL) and the IL-6/IL-10 ratio (>3.5) were shown to be as valuable in the diagnosis of NS as CRP. The interleukins with the highest specificity and sensitivity were IL-6 and the IL-6/IL-10 ratio at 94.1% and 100% respectively[41]. Another study recognised that distinct cut-off values must be used depending on neonatal age. It examined cut-off values of serum IL-6 at 80 pg/ml on day of life 1, 40 pg/ml on day of life 2–7 and 30 pg/ml after day of life 7[42]. A sensitivity of 75% and specificity of 81% for culture-confirmed sepsis was achieved, concluding that these IL-6 cut offs have a high accuracy for the detection of NS.

Interleukin-35

Interleukin-35 (IL-35) is an anti-inflammatory cytokine produced by immune cells including T-regulatory, dendritic, macrophages and monocytes as well as vascular endothelial, and smooth muscle cells during sepsis. Recent research aimed to assess the viability of IL-35 as a predictive biomarker for NS[43]. IL-35 levels were compared between neonates with and without sepsis, with septic neonates showing significantly higher IL-35 levels. The ROC curve analysis indicated an AUC of 89.5%, with an IL-35 cutoff >8.05 pg/ml showing 97% sensitivity[43].

Another study compared laboratory results between two groups: septic neonates and those unlikely to be infected with NS. Infected neonates had significantly higher levels of IL-35, PCT, CRP and white blood cell (WBC) counts compared to the likely uninfected group. IL-35 consistently showed higher levels in infected neonates across different time intervals. For predicting NS, IL-35 performed well with an AUC of 75.6%, while PCT, CRP, and WBC had lower AUC values[44]. These findings showcase the promise IL-35 holds as a predictive biomarker, however further research with larger sample sizes/ diverse populations is needed to confirm its utility, stability, and potential convenience for clinical diagnosis.

Angiopoietin

Prominent among the biomarkers studied in NS are angiopoietin-1 and -2 (Ang-1 and Ang-2). Ang-1 promotes vascular maturation and stability by binding to receptor Tie-2, a tyrosine kinase receptor primarily found in endothelial cells[45]. In general, Ang-1 can be viewed as a stabilising messenger, causing continuous Tie-2 phosphorylation, while Ang-2 acts as a destabilising messenger. Angiopoietins can directly stimulate both endothelial cells and neutrophils for an overall pro-inflammatory and pro-angiogenic response in sepsis. This balance between Ang-1 and Ang-2 is crucial in determining vascular development and maintenance[45].

Serum Ang-2 levels are associated with sepsis severity and are predictive of outcomes, however it is essential to quantify the reliability, cutoffs, and added value of Ang-2 over traditional markers before it can be used in clinical practice[9]. The angiopoietin-Tie-2 system shows some promise in sepsis diagnosis, but rigorous clinical studies are needed to confirm its utility as a biomarker, particularly in the ED, as testing is currently expensive as well as time consuming.

MicroRNA

MicroRNAs (miRNA) are small (20-24 nucleotides), endogenous non-coding RNA molecules[46-48]. If a miRNA is a perfect complement to its target mRNA, miRNA binds to the 3'-untranslated region of the target mRNA, leading

to destabilisation and degradation via deadenylation and capping[46-48]. If the miRNA is a partial complement, the molecule can inhibit translation, preventing conversion of mRNA to protein. Due to their precise intrusion into several molecular pathways and pathology-specific expression levels, miRNAs have been pinned as promising future diagnostic and therapeutic tools in disorders characterised by aberrant cellular signalling.

The literature directly implicates miRNAs in the modulation of NS pathology. MicroRNAs strongly regulate TNF pathways, one of the major signalling arms in sepsis[46]. Furthermore, stimulating macrophages and hepatic cells by bacterial lipopolysaccharide (LPS) produces increased miR-155 expression from these cell lines[46]. Similar experiments with LPS induction in monocyte cell lines have shown increased miR-150 and let-7a (a precursor miRNA) expression levels[46].

The structural stability and specificity suggest the clinical utility of miRNAs in the early diagnosis of NS. Furthermore, detection requires extremely low blood volumes which offers advantages in neonate diagnostics in the ED.

Initial work implicated several miRNAs as diagnostic and prognostic biomarkers in septic adult patients. However, the incomparable developmental and immunological states of neonate and adult septic patients does not make these results generalisable. Indeed, Cheng et al. have reported that the repertoire of miRNAs dysregulated in neonates is profoundly different to that in adult patients[49].

In neonates, miR-26a is found to be downregulated in the serum and blood mononuclear cells. Cheng et al. report that this change in miRNA expression is associated with IL-6 overexpression in septic neonates, especially those suffering from early onset NS[50]. Moreover, another study found that serum levels of both IL-6 and miR-26a may be indicative of the extent of inflammatory response to tissue injury[51].

Fatmi et al. studied miR-23b, a miRNA which plays a major role in attenuating the effects of pro-inflammatory cytokines, in both EOS and LOS patients. They found that EOS patients with positive haemoculture showed increased miR-23b levels, while both full-term and preterm LOS patients showed decreased levels[52]. The team also found that downregulation of miRNA-23b in NS has the potential as a prognostic biomarker, as decreased expression of miRNA was associated with neonatal death[52].

A recent meta-analysis has highlighted additional miRNAs worthy of future investigation[53]. El-Khazragy et al. highlight that miR-34a has a sensitivity of 89% and specificity of 97% for ruling out NS[53]. Downregulated miR-34a was also correlated with disease progression, while increased levels of miR-1 and miR-124 were associated with poor prognosis[53]. All three of these modulate aspects of the innate immune response such as the polarisation of the M1/M2 macrophage axis[53].

MicroRNA testing would be favourable in time-sensitive NS ED diagnoses, as the assay time has been estimated to be 2-2.5 hours[53]. The ease of use and the commendable specificity and sensitivity of the tests, further support the use of miRNAs in the early diagnosis of NS.

Overall, detection of these miRNAs in serum of patients may be a useful diagnostic and prognostic tool, possibly providing early diagnosis. A future research avenue could be to assess the practicality of using miRNA expression levels in guiding appropriate antibiotic prescription on a pathogen-specific basis.

Conclusion

While blood cultures play a useful diagnostic role, particularly in identifying the specific strain of bacteria, and thus guiding more targeted antibiotic therapy, other biomarkers should be used in adjunct. However, a combinational approach may be most useful in the diagnostic and prognostic roadmap to improving outcomes in NS. Exploration of the named biomarkers reveals intriguing findings related to vascular regulation and immune response during the neonatal phase of life.

Presepsin, a marker resistant to perinatal factors, exhibits rapid elevation upon bacterial infection, making it valuable in the ED. PCT responds swiftly to bacterial sepsis, showing a higher sensitivity EOS; this can help to ensure a definitive diagnosis, reducing hospital stays, antibiotic overuse and thus microbial resistance. The pronounced elevation of markers like IL-6 and Ang-2 levels in neonates with sepsis presents an intriguing prospect, potentially serving as a valuable biomarker for assessing sepsis severity and offering prognostic insights. MiRNAs, particularly miR-26a, miR-23b, and miR-34a, exhibit altered patterns in NS, and due to their specificity and ease of detection, they make for potential high yield markers in the ED.

While these biomarkers hold promise, further large-scale studies are needed before standardisation and recommendations for routine clinical practice are established. Overall, it is undeniable that these biomarkers would be a significant addition to our arsenal, enabling rapid diagnosis, accurate risk assessment, and improving prognostic outcomes. ◄

Declarations

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. The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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