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Peer Reviewer of *World Journal of Methodology*, Mohammad Sultan Khuroo, MD, DM, FRCP (Edin), FACP, Master American College of Physicians (MACP, Emeritus), Digestive Diseases Centre, Dr. Khuroo's Medical Clinic, Srinagar. khuroo@yahoo.com.

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EDITORIAL

Ethical framework for artificial intelligence in healthcare research: A path to integrity

Ahmad A Abujaber, Abdulqadir J Nashwan

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Ahmad A Abujaber, Department of Nursing, Hazm Mebaireek General Hospital (HMGH), Doha 3050, Qatar

Abdulqadir J Nashwan, Department of Nursing, Hamad Medical Corporation, Doha 3050, Qatar

Corresponding author: Abdulqadir J Nashwan, MSc, Research Scientist, Department of Nursing, Hamad Medical Corporation, Rayyan Road, Doha 3050, Qatar. anashwan@hamad.qa

Abstract

The integration of Artificial Intelligence (AI) into healthcare research promises unprecedented advancements in medical diagnostics, treatment personalization, and patient care management. However, these innovations also bring forth significant ethical challenges that must be addressed to maintain public trust, ensure patient safety, and uphold data integrity. This article sets out to introduce a detailed framework designed to steer governance and offer a systematic method for assuring that AI applications in healthcare research are developed and executed with integrity and adherence to medical research ethics.

Key Words: Artificial intelligence; Ethical framework; Healthcare research; Ethical Principles; Integrity; Patient safety

Core Tip: This editorial sets out to introduce a detailed framework designed to steer governance and offer a systematic method for assuring that artificial intelligence applications in healthcare research are developed and executed with integrity and adherence to medical research ethics.

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INTRODUCTION

The integration of artificial intelligence (AI) into healthcare research marks a pivotal shift towards groundbreaking advancements in diagnostics, treatment, and patient care management. This evolution, however, introduces a spectrum of ethical challenges that necessitate meticulous scrutiny and governance. At the heart of these challenges are concerns over privacy and confidentiality, as AI solutions require access to extensive patient data, raising significant risks to individual privacy[1]. The issue of informed consent also becomes more complex, as the applications of AI in healthcare research may extend beyond the scope of traditional consent frameworks, necessitating updated procedures that transparently communicate the potential uses of patient data[2,3].

Moreover, the inherent risk of bias in AI algorithms presents a critical ethical dilemma, with the potential to perpetuate existing disparities in healthcare outcomes[4]. Ensuring fairness and addressing biases is paramount to uphold ethical standards in AI-driven healthcare solutions. Transparency and explainability of AI decision-making processes are essential to maintain trust and accountability, particularly in a field that is sensitive such as healthcare[5]. The question of accountability for AI-driven decisions further complicates the ethical landscape[6], alongside concerns about equitable access to AI benefits, which could inadvertently widen health disparities[7].

Addressing these ethical challenges is crucial to leveraging AI in healthcare research responsibly. It requires a collaborative effort from researchers, ethicists, policymakers, and the broader healthcare community to develop a comprehensive ethical framework. Such a framework aims not only to mitigate risks but also to ensure that AI advancements contribute positively to patient care, uphold patients' rights, and promote equity. Although there is significant discussion in academic circles about the essential need for a robust ethical framework to oversee the integration of AI in healthcare research, current literature lacks a detailed framework that articulates foundational ethics and sets the operationalization guidelines and the implementation principles. Our research represents an innovative step toward creating a thorough and solid ethical structure. This proposed framework is designed to protect ethical integrity and ensure the highest ethical practices in AI healthcare research.

THE IMPERATIVE OF AN ETHICAL FRAMEWORK

The rapid advancement and integration of AI in healthcare research emphasizes the pressing need for an ethical framework to guide its application[8]. The absence of such a framework risks ethical lapses that could undermine public trust, compromise patient privacy, and exacerbate healthcare disparities. An ethical framework serves as a compass, guiding researchers and practitioners in navigating the complex moral terrain of AI in healthcare[9].

Adopting a robust ethical framework offers several remedies to these challenges. First, it ensures that privacy and confidentiality are paramount, safeguarding patient data against misuse. By establishing clear guidelines for data handling, the framework can mitigate risks associated with data breaches and unauthorized access. Second, it enhances informed consent processes, ensuring that participants are fully aware of how their data will be used, including potential AI applications, thus respecting their autonomy[10].

Moreover, an ethical framework addresses biases in AI algorithms, promoting fairness in healthcare outcomes[11]. It mandates regular audits of AI systems for bias and requires the implementation of corrective measures when disparities are identified. Transparency and explainability become foundational, with AI systems designed to provide understandable outputs, thereby fostering trust among patients and healthcare providers[10].

Finally, an ethical framework ensures accountability and equitable access to AI-driven healthcare innovations. It delineates responsibilities among AI developers, healthcare providers, and policymakers, ensuring that those impacted by AI decisions have recourse[12]. By prioritizing equitable access, the framework also works to prevent the widening of health disparities, making the benefits of AI in healthcare research accessible to all[10].

PROPOSED ETHICAL FRAMEWORK FOR AI IN HEALTHCARE RESEARCH

The proposed framework, as illustrated in Figure 1, is centered around the four key ethical principles in medicine, further supported by actionable guidelines to operationalize these principles in practical research settings. The framework is visualized in a diagram that illustrates its multifaceted approach, focusing on the core ethical principles of respect for autonomy, beneficence, non-maleficence, and justice as its core pillars.

Core ethical principles

The core ethical principles guiding AI in healthcare research are deeply rooted in the foundational principles of medical research ethics. These principles are not only relevant but essential in ensuring that AI technologies are developed and used in ways that align with the ethical conduct of medical research. Here's how these core principles relate to medical research ethics:

Respect for autonomy: In medical research, respecting autonomy involves acknowledging and upholding the rights of participants to make informed decisions about their involvement. This principle is crucial in AI healthcare research, especially in the context of informed consent. It emphasizes the importance of ensuring that individuals are fully informed about how their data will be used in AI applications, reflecting their autonomy in the decision-making process.

Figure 1 The proposed proposed ethical framework for artificial intelligence in healthcare research. AI: Artificial intelligence.

Beneficence: Beneficence in medical research ethics refers to the obligation to maximize benefits and minimize harms to research participants. In the context of AI, this principle mandates that the development and application of AI technologies should aim to improve healthcare outcomes and patient care, ensuring that the benefits of AI advancements are realized and maximized in clinical settings.

Non-maleficence: The principle of non-maleficence, or "do no harm," is vital in medical research, emphasizing the importance of avoiding harm to participants. In AI healthcare research, this translates to ensuring that AI systems do not inadvertently cause harm, such as through biases in algorithms that could lead to incorrect diagnoses or treatment recommendations. It highlights the need for rigorous testing and validation of AI models to prevent potential adverse outcomes.

Justice: Justice in medical research ethics focuses on ensuring equitable access to the benefits of research and fair distribution of risks and burdens. When applied to AI in healthcare, this principle highlights the need to address and mitigate healthcare disparities that AI solutions might exacerbate. It calls for the equitable development and deployment of AI solutions, ensuring that all patient populations can benefit from AI advancements without widening existing healthcare gaps.

Integrating these core ethical principles into the development and application of AI in healthcare research ensures that AI solutions are not only innovative but also ethically responsible. It aligns AI advancements with the longstanding commitments of medical research to respect human dignity, promote well-being, avoid harm, and distribute healthcare benefits justly among all segments of the population. This integration is crucial for maintaining trust in AI applications in healthcare and ensuring that these powerful tools serve the collective good of society.

Operational guidelines

Building upon the core ethical principles, operationalization defines the precise measures and indicators for ethical principles, translating abstract concepts into quantifiable criteria. The following operational guidelines are designed to practically guide the seamless integration of the core ethical principles into every phase of AI development and application in healthcare. However, the effective institutionalization of these guidelines hinges on the creation of precise, quantifiable, and observable metrics that capture the practical implementation of these ethical principles. This crucial task is entrusted to research institutions and Institutional Review Boards (IRBs), underlining their role in ensuring that ethical considerations are integrated consistently and effectively across the spectrum of AI healthcare initiatives.

Transparency and explainability: AI systems should be transparent in their operations, with mechanisms in place to explain decisions to both practitioners and patients.

Privacy and data protection: Strict protocols that adhere to the relevant laws and regulations such as the Health Insurance Portability and Accountability Act (HIPAA) must be established to protect patient data, respecting privacy, and confidentiality throughout the research process.

Inclusive design and bias mitigation: AI technologies should be designed with diverse populations in mind, actively working to mitigate biases in datasets and algorithms.

Stakeholder engagement: Patients, healthcare providers, ethicists, and policymakers should be involved in the development and implementation of AI applications to ensure a wide range of perspectives are considered.

Implementation guidelines

Implementing the ethical framework for AI in healthcare research is a comprehensive approach that embeds fundamental ethical principles throughout every phase of AI development and deployment. This phase transitions from theoretical planning to tangible action, executing the strategies, plans, or policies established during the operationalization phase. Implementation entails the application of operationalized components to realize specific goals, encompassing all practical aspects of enacting a plan. This includes allocating resources, organizing schedules, and conducting the activities necessary to bring theoretical concepts and strategies to fruition.

This phase requires active collaboration among various stakeholders, including researchers, clinicians, patients, ethicists, and policymakers[8]. Here are key steps to effectively implement the ethical framework:

Multi-disciplinary collaboration: The multidisciplinary collaboration entails: (1) Establishing interdisciplinary teams that include ethicists, data scientists, healthcare professionals, and preferably a patient representative to guide the ethical development and deployment of AI solutions[13]; and (2) Facilitating regular discussions and workshops to address ethical concerns and integrate diverse perspectives into AI research and development processes.

Education and training: Education and training include: (1) Developing educational programs and resources for AI researchers and healthcare professionals that focus on the ethical implications of AI in healthcare. Abujaber *et al*[1] proposed that educational institutions, particularly those specializing in health-related fields, should begin integrating AI into their curricula during the collegiate years. Such early exposure to AI is recommended to ease future acceptance and adoption among students entering healthcare professions[1]; and (2) Including modules on ethical decision-making, bias recognition, and mitigation strategies in AI development curricula.

Policy development and regulatory compliance: This can be achieved by two steps: (1) Working with regulatory bodies to ensure that policies and guidelines for AI in healthcare research reflect the core ethical principles[13]; and (2) Encouraging the adoption of standards and best practices that promote transparency, accountability, and equity in AI applications.

Ethical review and oversight: This requires: (1) Implementing ethical review processes specifically tailored to AI projects in healthcare research, akin to traditional human subjects' research oversight; and (2) Establishing ethics committees or boards with expertise to evaluate AI projects, focusing on the potential risks, benefits, and ethical considerations.

Public and stakeholder engagement: The advocacy for integrating AI into medical research is driven by the objective to fully harness its potential in improving patient care, providing support to patients' families, and benefiting the wider community. Consequently, the successful implementation of AI depends on the active involvement of patients, their families, and other key stakeholders. Engagement should encompass: (1) Collaborating with patients, the public, and stakeholders *via* consultative and participatory design methods to solicit feedback on the development of AI and its potential impacts[14]; and (2) Promoting transparency by publicly sharing information about AI projects, including objectives, methodologies, and ethical considerations[15].

Continuous monitoring and evaluation: This involves: (1) Monitoring the outcomes of AI applications in healthcare research continuously to identify unforeseen ethical issues or adverse effects; and (2) Implementing mechanisms for ongoing evaluation and adaptation of AI technologies, ensuring they remain aligned with ethical principles and societal values[16].

Feedback loop: This requires: (1) Creation of a feedback loop that allows for the continuous integration of lessons learned from the implementation of AI technologies back into the ethical framework; and (2) Refinement and updating the ethical framework regularly based on new insights, technological advancements, and evolving societal norms.

Implementing this ethical framework is an ongoing process that requires commitment, transparency, and adaptability. By taking these steps, stakeholders can ensure that AI in healthcare research is conducted with the highest ethical standards, ultimately benefiting society while safeguarding individual rights and promoting equity.

ETHICAL ISSUES AND PROPOSED SOLUTIONS

Addressing how AI can potentially compromise patient confidentiality and the measures that can be implemented to safeguard sensitive health information.

Discussion on the risks of bias in AI algorithms, its impact on treatment and diagnosis outcomes across different demographics, and strategies for mitigation.

Exploring the complexities of informed consent when using AI, including the use of patients' data for training AI systems.

Clarifying who is held accountable when AI systems make errors or cause harm, and how liability is managed in the deployment of AI technologies in healthcare.

Proposing specific guidelines for data management that comply with existing regulations and ethical standards, like HIPAA, to ensure data privacy and security.

Providing clear guidelines for maintaining transparency in the operations of AI systems and the logic behind AI decision-making processes.

Recommending strategies for designing AI systems that are inclusive and equitable, ensuring fair representation and treatment of all patient groups.

Outlining the role of IRBs and other ethics committees in ongoing oversight, including regular ethical reviews and the monitoring of AI systems post-deployment to quickly identify and address new ethical issues.

CONCLUSION

As AI technologies become increasingly integrated into healthcare research, establishing ethical foundations is imperative to ensure these innovations serve the public good. While the proposed ethical framework serves as a foundational step towards guiding AI applications in healthcare research, it is evident that further refinement is essential to align it seamlessly with the existing medical research governance systems. Achieving higher ethical standards in AI healthcare research relies not only on the institutional adoption of this framework but also on its continuous enhancement to improve practical implementation. Success in this domain is contingent upon both the robust integration of the framework within institutional practices and the ongoing efforts to increase its operational effectiveness.

FOOTNOTES

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Country of origin: Qatar

ORCID number: Ahmad A Abujaber 0000-0002-8704-4991; Abdulqadir J Nashwan 0000-0003-4845-4119.

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REVIEW

COVID-19 mutations: An overview

Malay Sarkar, Irappa Madabhavi

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Malay Sarkar, Department of Pulmonary Medicine, Indira Gandhi Medical College, Shimla 171001, Himachal Pradesh, India

Irappa Madabhavi, Department of Medical and Pediatric Oncology and Hematology, J N Medical College, and KAHER, Belagavi, Karnataka 590010, India

Irappa Madabhavi, Department of Medical and Pediatric Oncology and Hematology, Kerudi Cancer Hospital, Bagalkot, Karnataka 587103, India

Corresponding author: Irappa Madabhavi, MBBS, MD, DM, ECMO. Consultant Physician-Scientist, Department of Medical and Pediatric Oncology and Hematology, J N Medical College, and KAHER, Belagavi, Karnataka 590010, India. irappamadabhavi@gmail.com

Abstract

The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) belongs to the genus Beta coronavirus and the family of Coronaviridae. It is a positive-sense, non-segmented single-strand RNA virus. Four common types of human coronaviruses circulate globally, particularly in the fall and winter seasons. They are responsible for 10%-30% of all mild upper respiratory tract infections in adults. These are 229E, NL63 of the Alfacoronaviridae family, OC43, and HKU1 of the Betacoronaviridae family. However, there are three highly pathogenic human coronaviruses: SARS-CoV-2, Middle East respiratory syndrome coronavirus, and the latest pandemic caused by the SARS-CoV-2 infection. All viruses, including SARS-CoV-2, have the inherent tendency to evolve. SARS-CoV-2 is still evolving in humans. Additionally, due to the development of herd immunity, prior infection, use of medication, vaccination, and antibodies, the viruses are facing immune pressure. During the replication process and due to immune pressure, the virus may undergo mutations. Several SARS-CoV-2 variants, including the variants of concern (VOCs), such as B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617/B.1.617.2 (Delta), P.1 (Gamma), and B.1.1.529 (Omicron) have been reported from various parts of the world. These VOCs contain several important mutations; some of them are on the spike proteins. These mutations may lead to enhanced infectivity, transmissibility, and decreased neutralization efficacy by monoclonal antibodies, convalescent sera, or vaccines. Mutations may also lead to a failure of detection by molecular diagnostic tests, leading to a delayed diagnosis, increased community spread, and delayed treatment. We searched PubMed, EMBASE, Covariant, the Stanford variant Database, and the CINAHL from December 2019 to February 2023 using the following search terms: VOC, SARS-CoV-2, Omicron, mutations in SARS-CoV-2, *etc.* This review discusses the various mutations and their impact on infectivity, transmissibility, and neutralization efficacy.

Key Words: Variant of concern; SARS-CoV-2; Omicron; N501Y mutation; E484K mutation

Core Tip: The severe acute respiratory syndrome coronavirus-2 virus is constantly evolving because to natural immunity and vaccine-induced immunity which exert continual immunological pressure, resulting in the generation of newer variants and numerous new mutations. This study detailed the many variants of concern (VOCs), including their transmissibility, severity, and immune-evasion capacities. We have also discussed several key mutations and their consequences. The tables summarized the major points of the paper and provided a full discussion of the important mutations found in these VOCs. Readers will benefit from our article's concise overview of these areas.

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INTRODUCTION

Variants are coronaviruses that have the same inherited set of very distinctive mutations[1]. It is worth noting that RNA viruses tend to have higher mutation rates than DNA viruses, and single-stranded viruses mutate quicker than double-stranded viruses[2]. However, the rate of mutations among the coronaviruses is lower than that of most RNA viruses. The severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) acquires 2-3 single-nucleotide (nt) mutations in its genome per month. This is half the rate of influenza (4 nt/month) and one-fourth the rate of human immunodeficiency virus (8 nt/month) mutations[3,4]. The slow rate of mutation may be explained by the existence of a novel 3′-to-5′ exoribonuclease (ExoN) in nsp14, which serves as a proofreader and corrects some replication errors[5]. Genetic inactivation of this ExoN function causes a 15- to 20-fold rise in mutation rates[6]. When variants with various mutations infect the same host, they accumulate mutations and create diversity *via* recombination[7-9]. Human hosts may also contribute to the diversity *via* host-mediated RNA editing[10,11]. It is important to know that not every mutation will have a long-lasting effect on the virus. Typically, synonymous mutations are neutral, whereas non-synonymous mutations are persistent[12]. Wang *et al*[13] observed that in SARS-CoV-2, non-synonymous mutations are 14 times less likely to persist as weak deleterious mutations are slowly eliminated[13]. Mutations may increase infectivity, transmissibility, disease severity, and decreased neutralization efficacy by monoclonal antibodies (mAbs) and convalescent sera, as well as lower vaccine efficacy (VE). Moreover, a few variants may be responsible for negative results on the diagnostic tests. For example, with the Alpha and Omicron variants, there could be "*S*" gene target failure (SGTF)[14-16]. Delay in diagnosis increases the likelihood of viral transmission. The surface transmembrane spike (S) protein consists of S1 and S2 subunits. The S1 subunit has the N-terminal domain (NTD) and a receptor-binding domain (RBD) and it mediates the critical step of viral entry into the cell *via* interacting with the angiotensin-converting enzyme 2 (ACE2) receptor of the host cell[17]. The S1 subunit is also the primary target of neutralizing antibodies upon infection and various therapeutic and vaccines [18]. S2 mediates fusion of the viral and cellular membranes[19]. The RBD must be in an upright position to bind with ACE2 receptors and initiate the viral entry process. It is followed by the cleavage of S1/S2, which helps in membrane fusion (Figure 1A). The SARS-CoV-2 also enters in the cell by endosomal/lysosomal pathway. Inside the cell, the virus undergoes replication, translation, assembly, and exocytosis followed by viral release (Figure 1B). The most common site of mutation in SARS-CoV-2 is the spike protein, but it may involve other proteins as well. The diagnostic assays for SARS-CoV-2 are based on the two most abundant and immunogenic viral proteins, such as spike or nucleocapsid (N) proteins[20]. The spike protein contains sequences that are unique to the SARS-CoV-2 virus, thereby reducing the risk of cross-reactivity. However, spike protein has the highest potential to undergo mutation, and it has the potential to cause false-negative tests on immunoassays that are based only on detecting spike protein. Since N protein is less susceptible to mutations, it is the best target for developing diagnostic assays[20]. Recently, several variants of concern (VOCs) have been de-escalated. It will provide updated knowledge on the de-escalated status of various VOCs, subcategories, and VE. A detailed evaluation of the existing mutations and emerging mutations has been of immense help in studying their impact on transmissibility, severity, neutralization potency, and VE. This narrative review will provide an updated version of SARS-CoV-2 VOCs, associated mutations, the effect of mutations, and the present status of the VOCs in detail. Understanding the biological characteristics of the mutations will guide us in the surveillance, prevention, and control of coronavirus disease 2019 (COVID-19) infection.

SARSCOV2 VARIANT'S NOMENCLATURE SYSTEM

The SARS-CoV-2 interagency group (SIG) of the United States Department of Health and Human Services has classified the newly developing variations of SARS-CoV-2 into many groups. The SIG is responsible for better coordination among

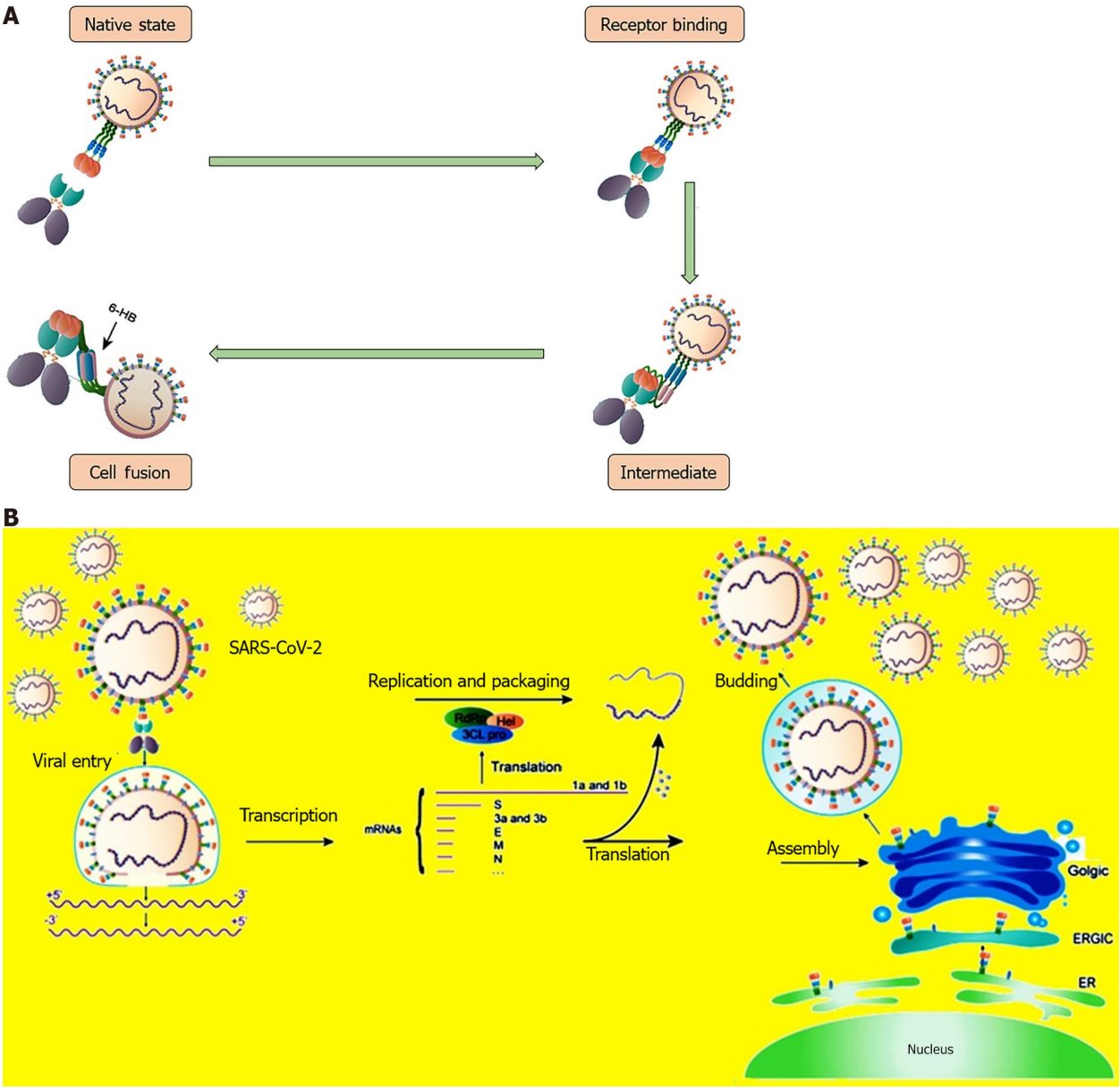

Figure 1 Showing the S protein mediated binding, fusion and various steps of severe acute respiratory syndrome coronavirus-2 infection cycle in the humans. A: Showing the S protein mediated binding and fusion with human cell; B: Showing the various steps in the severe acute respiratory syndrome coronavirus-2 infection cycle in human. It includes viral cellular entry, transcription, replication and packaging, translation, assembly within cellular organelle. SARS-CoV-2: Severe acute respiratory syndrome coronavirus-2; ER: Endoplasmic reticulum; ERCIC: ER-to-Golgi intermediate compartment; RdRP: RNA-dependent RNA polymerase; 3CLpro: 3C-like proteinase; Hel: Helicase.

various United States departments and agencies, rapid characterization of emerging variants, and actively monitoring their potential impact on vaccines, therapeutics, and diagnostics[21]. Due to the lack of a standardized system, various nomenclature systems are in use. The Global Initiative on Sharing Avian Influenza Data, Nextstrain, and Phylogenetic Assignment of Named Global Outbreak (PANGO) are the most commonly used nomenclature systems by the scientific community worldwide[22]. The PANGO lineage system contains an alphabetical prefix and a suffix containing up to three numbers separated by periods indicating sub-lineages (such as B.1.1.7). Starting from high impact to low impact, SARS-CoV-2 variants may be classified as variants of high consequence, VOCs, variants of interest, and alerts for further monitoring. The VOC is characterized by increased transmissibility, a severe disease associated with an increased rate of hospitalizations or deaths, a significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures[23].

TRANSLATIONAL SCIENCE

The COVID-19 pandemic has demonstrated the importance of translational research in pandemic control. Translational research encompasses a wide range of disciplines, including diagnostics, newer drug development, pathogenesis,

epidemiology, and vaccine development. The COVID-19 pandemic has changed the traditional approach to clinical research[24].

NATURAL SELECTION AND IMMUNE IMPRINTING

Two phenomena play a significant role in the natural history of SARS-CoV-2 virus evolution. These include natural selection and immune imprinting. Animal experiments have confirmed the existence of natural selection. Lei *et al*[25] investigated the composition and codon use of the SARS-CoV-2 virus in infected humans and animals[25]. They reported the maximum mutations in mink. SARS-CoV-2 in mink showed that substitutions of cytidine contributed to approximately 50% of substitutions. The corresponding figure for other animals was 30%. However, the incidence of adenine transversion in SARS-CoV-2 in other animals is three times higher than in mink. They also found lower adaptability than humans in all other animals except for mink. Furthermore, a binding affinity analysis revealed that the spike protein of the SARS-CoV-2 variant in mink had a greater preference for binding with the mink receptor ACE2 than the human receptor, particularly with the mutations Y453F and F486L in mink SARS-CoV-2, which improved the binding affinity for the mink receptor. This study demonstrates that SARS-CoV-2's natural history in mink includes both natural selection and host adaptation. Similarly, Fu *et al*[26] showed that natural selection had a stronger influence on some SARS-CoV-2 sequences than mutational pressure[26]. The Y453F and N501T mutations in mink SARS-CoV-2 increased viral spike binding to the mink receptor. It confirmed the role of these mutations in natural selection and viral fitness. Natural selection favors the strains with beneficial mutations and reduces the number of strains with deleterious mutations[27]. However, it is still unclear whether natural selection occurred first in an animal host before zoonotic transfer or whether natural selection occurred in humans after zoonotic transfer[28]. Rubio-Casillas *et al*[29] coined the term "intermittent virulence", which is basically an evolutionary equilibrium between transmissibility and virulence[29]. They considered this phenomenon to be due to natural selection. Habib *et al*[30] scanned the RBD of the Omicron spike protein for adaptive evolution based on a public database in Bangladesh[30]. It was reported that the adaptive mutations in the RBD domain were characterized by a non-synonymous to synonymous nt substitution rate of more than one. This indicates a positive selection. Some of the adaptive sites mediate increased viral fitness. Immune imprinting is the mechanism by which memory B lymphocytes induced by an initial viral infection prevent the development of B cells in response to a subsequent infection with a novel but related virus[31]. Chemaitelly *et al*[32] conducted a retrospective cohort study in Qatar to compare the incidence of SARS-CoV-2 reinfection in persons who had received primary-series (two-dose) vaccination, no vaccination, or booster (three-dose) vaccinations[32]. They found that a history of primary-series immunization enhanced immune protection against omicron reinfection, whereas a history of booster vaccination compromised protection against omicron reinfection. In the future, a study elucidating the pathogenetic mechanism behind the phenomenon of immune imprinting may provide useful insights for creating a more effective vaccine against the SARS-CoV-2 virus. In addition, we should not forget the short-term public health benefits of vaccination.

VOC

Several VOCs have been identified, and they differ from one another in terms of infectivity, transmissibility, severity, therapeutic efficacy, and neutralization efficacy by mAbs, convalescent sera, or vaccines. These are B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617/B.1.617.2 (Delta), P.1 (Gamma), and B.1.1.529 (Omicron). The European Center for Disease Prevention and Control declared a new category in July 2021 as de-escalated variants. These VOCs have been de-escalated because they are either not circulating or, if they are, have no epidemiological impact. Moreover, they are not associated with any concerning properties[33].

Alpha (B.1.1.7 lineage) VOC

The B.1.1.7 variant was the first VOC to be detected in southeast England in September 2020. It eventually became the dominant variant in the United Kingdom and many other countries due to its increased transmissibility[33]. The B.1.1.7 variant was found to be 50%-75% more transmissible than the origin lineage, with a R0 value that was 1.75 times higher [34]. Another feature is the increased disease severity compared to the pre-existing SARS-CoV-2 variants of that time. Davies *et al*[35] had shown that the hazard of death with SGTF was 55% (95%CI: 39%–72%) higher than that in cases without SGTF after adjustment for age, sex, ethnicity, deprivation, care home residence, local authority of residence, and test date[35]. The B.1.1.7 variant's higher transmissibility is due to the presence of the N501Y mutation and Deletion69/Deletion70, which increase binding affinity to ACE2[36,37]. Other characteristics include SGTF due to mutations in the *S* gene and no change in susceptibility to monoclonal antibody therapy such as Bamlanivimab-etesevimab, casirivimab-imdevimab, and sotrovimab[38-40]. However, E484K and/or other NTD mutations (especially deletions) may result in a considerable reduction in neutralizing efficacy[41]. The ChAdOx1 nCoV-19 (AstraZeneca) vaccine showed an efficacy of 70.4%[42]. The first and second doses of the BNT162b2 vaccine (Pfizer–BioNTech) reported 48.7% and 93.7% effectiveness, respectively[43]. The reported efficacy of two doses of the mRNA-1273 (Moderna) vaccine was 98.4%[44].This variant has been de-escalated[33].

Beta (B.1.351) VOC

Tegally *et al*[45] detected this variant, also known as 501Y.V2, in late 2020 in the Eastern Cape, South Africa[45]. The beta

variants also show increased transmissibility, similar to the B.1.1.7 variants. In comparison to the alpha and gamma versions, this variant increased the likelihood of hospitalization, intensive care unit (ICU) admission, and mortality. However, it causes less severe disease than the delta variant[46]. These variants also show immune-evasion properties. There was a 45-fold decrease in susceptibility to Bamlanivimab-etesevimab therapy; however, casirivimab-imdevimab and sotrovimab retained susceptibility[38-40]. Furthermore, beta versions demonstrate lower neutralization by convalescent and post-vaccination sera[47]. In a systematic review and meta-analysis, Zeng *et al*[48] assessed 11 COVID-19 vaccines (BNT162b2, mRNA-1273, ChAdOx1, Ad26.COV2.S, BBV152, CoronaVac, NVX-CoV2373, BBIBP-CorV, CVnCoV, SCB-2019, and HB02) and reported full vaccination efficacy against Alpha, Beta, Gamma, Delta, and Omicron variants of 88.0% (95%CI: 83.0%–91.5%), 73.0% (95%CI: 64.3%–79.5%), 63.0% (95%CI: 47.9%–73.7%), 77.8% (95%CI: 72.7%–82.0%), and 55.9% (95%CI: 40.9%–67.0%), respectively[48]. The efficacy of booster vaccination was higher against Delta and Omicron variants, 95.5% (95%CI: 94.2%–96.5%) and 80.8% (95%CI: 58.6%–91.1%), respectively. They also reported a higher efficacy of mRNA vaccines (mRNA-1273/BNT162b2) against VOC over others.

Gamma (P.1; GR/501Y.V3)

The P.1 variant was first reported from Japan on January 6, 2021, by four people who had arrived in Tokyo after visiting Amazonas, Brazil[49]. Faria *et al*[50] further published the genomic and epidemiological analysis of this Brazilian variant from Manaus[50]. They reported 17 mutations in the P.1 variants, including three in the spike protein RBDs (K417T, E484K, and N501Y). These mutations caused enhanced binding to the human ACE2 receptor. The P.1 variant is 1.7 to 2.4 times more transmissible than the previous (non-P.1) infection. Infection with P.1 is also 1.2 to 1.9 times more likely to cause mortality than previous lineages[50]. This variant also possesses immune evasion properties. Although the P.1 variant retained susceptibility to Casirivimab-imdevimab and Sotrovimab, there was a 511-fold decrease in susceptibility to Bamlanivimab-etesevimab[38-40]. This variant has been deescalated. Full vaccination efficacy against the gamma variants was 63.0%[48].

Delta (G/478 K.V1; B.1.617.2)

The B.1.617.2 variant was first identified in India in October 2020 and quickly became the dominant variant in India and globally until the emergence of the Omicron variant. This variant was 40%-60% more transmissible than the B.1.1.7 variant and almost twice as transmissible as the original Wuhan strain[51]. The B.1.617 variant has three sublineages: B.1.617.1, B.1.617.2, and B.1.617.3. The B.1.617.2 variants show increased transmissibility and replication advantages. This variant shows 1260-fold higher viral loads than those for the 2020 infections with clade 19A/19B viruses. It makes the person more infectious[52]. Compared to the B.1.1.7 cases, the B.1.617 variant is associated with an increased severity of the disease[53,54] and an increased risk of hospitalization[54,55]. The common signature mutations located in the spike protein are D111D, G142D, L452R, E484Q, D614G, and P681R. The L452R, E484Q, and P681R mutations contribute to increased transmissibility, and the E484Q and P681R mutations influence antibody binding. Neutralization by mAbs is affected minimally. Although there is a moderate reduction in VE against symptomatic COVID-19 infection, efficacy against severe disease and hospitalization showed no significant impact[47]. The 2-dose mRNA-1273 vaccine showed 86.7% (95%CI: 84.3%- 88.7%) efficacy against infection and 97.5% (95%CI: 92.7%-99.2%) efficacy against hospital admission with the B.1.617.2 variant[44]. However, VE decreased from 94.1% at 14-60 d after immunization to 80.0% at 151-180 d following vaccination. The efficacy of 2-doses of BNT162b2 and ChAdOx1 nCoV-19 vaccines was 88.0% (95%CI: 85.3%-90.1%) and 67.0% (95%CI: 61.3%-71.8%) among those with the delta variant, respectively[43]. Effectiveness after one dose of vaccine with both BNT162b2 and ChAdOx1 nCoV-19 vaccine was notably lower among persons with the delta variant (30.7%; 95%CI: 25.2%- 35.7%).

Omicron (B.1.1.529 lineage) variant

The Omicron variant was initially reported from Botswana and then from South Africa. Very soon, it became the dominant variant globally. The World Health Organization classified it as VOC on November 26 and named it Omicron [56]. Subsequently; several sublineages of the SARS-CoV-2 variant were identified. These are BA.1, BA.2, BA.4/BA.5, BA.4.6 (BA.4), BA.2.75.2 (BA.2), BQ.1/BQ.1.1 (BA.5), and XBB/XBB.1/XBB.1.5 (BA.2.10.1 and BA.2.75 recombinant) sublineages[47]. TheBA.4 and BA.5 sublineages have identical spike proteins similar to BA.2 except for the addition of 69-70 deletions, L452R, F486V, and the wild-type amino acid at Q493[57]. However, other sublineages differ from the others by at least one spike protein mutation[58]. Typical features of Omicron variants are high transmissibility, increased risk of reinfection or breakthrough infection, less severe disease compared to delta variants, and reduced or absent neutralization efficacy by vaccines and monoclonal antibody therapies. The Omicron variant is heavily mutated, as it contains up to 59 mutations in its genome, including 36 occurring within the spike protein and more than 30 involving the RBD[58, 59]. The Omicron variant has a replication advantage over the B.1.617.2 variant, with the basic reproduction number (R_0) exceeding 3[60,61]. The high R0 is the result of both higher transmissibility and immunological evasion. Compared to the earlier surge, hospitalized patients with the B.1.1.529 variant in Tshwane, Gauteng Province, South Africa, showed lower rates of ICU admissions (1% *vs* 4.3%, $P < 0.00001$), in-hospital death (4.5% *vs* 21.3%, $P < 0.00001$), and length of hospital stay (4.0 d *vs* 8.8 d)[62]. Omicron sublineages show immune evasion properties. Yue *et al*[62] reported that Omicron subvariant XBB.1.5 was more transmissible than other XBB sublineages[62]. This subvariant XBB.1.5 has an additional Ser486Pro substitution. The authors demonstrated a higher ACE2-receptor binding affinity and significant immune evasion in convalescent plasma. Moreover, Bebtelovimab showed no neutralization effect against the XBB.1/XBB.1.5 subvariant[63]. Similarly, Sotrovimab, Tixagevimab-cilgavimab, and Casirivimab-imdevimab remain inactive against the XBB/XBB.1/XBB.1.5 sublineages[47]. The mAbs resistant in B.1.1.529 variants may be explained by the presence of the following mutations: K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, S371L, and Y505H,

which are located within or close to the epitopes targeted by these antibodies. The Omicron variety (B.1.1.529 lineage) also has SGTF, which causes a delay in diagnosis and increases the risk of infection transmission. However, the BA.2 lineage does not show SGTF due to a lack of deletions in positions 69-70[64]. The above-mentioned monoclonal antibody cocktails should not be used against the B.1.1.529 variants[65]. Mass vaccination is a crucial public health intervention that lowers COVID-19-related hospitalization and mortality. However, the duration of protection wanes over time. Wu *et al*[66] in a meta-analysis, studied the long-term efficacy of COVID-19 vaccinations against infection, hospitalization, and mortality up to 307 d after completion of the primary vaccination series and 139 d after a first booster vaccination[66]. They reported a vaccine effectiveness of 83% against infection, 92% against hospitalization, and 91% against mortality after the primary COVID-19 vaccination. However, the efficacy decreased over time. The VE against the omicron sublineages was 61% and 71% against infection and hospitalization, respectively. However, a booster dose increased the vaccine effectiveness against the omicron variant to 67% against infection and 89% against hospitalization. Andrews *et al* [67] reported a decreased efficacy of the vaccine against the omicron variant compared to the delta variant[67]. The two doses of ChAdOx1 nCoV-19 (AstraZeneca) showed no efficacy against the symptomatic disease caused by the Omicron. The VE of 2-dose BNT162b2 doses and the mRNA-1273 vaccine were 65.5% (95%CI: 63.9%-67.0%) and 75.1% (95%CI: 70.8%-78.7%), respectively. However, efficacy decreases over time. Among patients who received ChAdOx1 nCoV-19 as the primary vaccine, a BNT162b2 and mRNA-1273 booster dose increased the efficacy to 62.4% (95%CI: 61.8%-63.0%) and 70.1% (95%CI: 69.5%-70.7%), respectively. Booster doses are required to mount a more appropriate immune response against omicron infection[46]. Among patients who received BNT162b2 as primary vaccine, a BNT162b2 booster dose increased the efficacy to 67.2% (95%CI: 66.5%-67.8%). The BNT162b2 vaccine showed an efficacy of 70% against hospitalization[68]. Table 1 shows the characteristic features of various VOCs.

COMMON MUTATIONS AND THEIR IMPACTS

D614G mutation

Genomic surveillance of SARS-CoV-2 during the first year of the COVID-19 pandemic revealed that the D614G mutation in the spike glycoprotein (Spike protein) was the predominant mutation in February 2020[69,70]. Later on, it spreads globally. The D614G mutation is caused by an aspartic acid-to-glycine substitution at position 614 of the spike glycoprotein. The D614G change is also associated with three other mutations: A, C-to-T mutation in the 5′ untranslated region (5′-UTR), a silent C-to-T mutation at position 3037, and a C-to-T mutation at position 14408, which causes an amino acid change in RNA-dependent RNA polymerase (RdRp)[70]. The D614G-mutated variants almost always carry these three mutations. Remdesivir targets the RdRp enzyme. Plante *et al*[71] examined the replication kinetics of the D614G variants in human lung epithelial cells (Calu-3 cells) and primary human airway tissues[71]. They found 2.4-fold more infectious virus at 36 hpi, indicating that the D614G mutation enhanced viral replication. Similarly, the golden Syrian hamster model infected with the D614G mutation produced higher infectious viral titers in the nasal washes and trachea but not in the lungs[71,72]. As a result, the D614G mutation may enhance viral loads in COVID-19 patients' upper respiratory tracts, increasing transmission. Korber *et al*[70] reported a lower real-time reverse transcription–polymerase chain reaction assay cycle threshold, which suggests higher viral loads and high infectivity[70]. However, the mechanism underlying improved replication fitness is unclear. Few studies have reported that increased cleavage efficiency of the spike protein into S1/S2 influences the SARS-CoV-2 infection[73,74]. However, Plante *et al*[71] observed no substantial differences in spike cleavage between the D614 and G614 virions, indicating that the enhanced infectivity is unlikely due to a D614G-mediated spike cleavage difference[71]. Another mechanism could be the disruption of the interprotomer latch between S1 and S2. Normally, the carboxyl groups in D614 form a hydrogen bond with the hydroxyl group in Thr859 across the S1/S2 interface[70]. The cryo-EM studies had shown that D614G disrupts the interprotomer latch between D614 in S1 and T859 in S2 and promotes the RDB domain to an "up" or open conformation and a higher chance of binding with the human ACE2 receptor. The ratio of closed and open conformation in D614 and G614 is 82% and 18%, and 42% and 58%, respectively[75]. Kannan *et al*[76] suggested that D614G alone would not be able to explain the high infectivity of the SARS-CoV-2 virus, and other coexistence mutations such as P323L (nsp12) and C241U (5′-UTR) and nsp mutations may also contribute to the infectivity[76]. D614G, by increasing the number of spike proteins per virion, may also be responsible for the increased infectivity[77,78]. However, since the 614 position lies outside the RBD, this mutation does not alter the affinity of spike protein to ACE2. Zhang *et al*[79] hypothesized that increased stability of the S-trimer in the presence of the D614G mutation may explain the enhanced infectivity as the S1 subunit dissociates more readily from the virus with an aspartic acid residue at position 614 than the virus having glycine at position 614[79]. The D614G mutation has been detected in B.1.1.7, B.1.351, P.1, and B.1.617.2 and B.1.1.529 lineages, indicating a transmission advantage of this mutation. However, it does not cause immune escape. Garcia-Beltran *et al*[80] had shown that the sera from convalescent individuals showed effective cross-neutralization of both wild type and D614G variants[80].

N501Y mutation

It includes replacing the amino acid asparagine (N) with tyrosine (Y) at position 501. The N501Y mutation has been identified in the B.1.1.7, B.1.351, P.1, and B.1.1.529 lineages. This mutation can alternatively be represented as S: N501Y, indicating that it occurs in the spike protein. The N501Y mutation is responsible for higher binding affinity to human ACE2 receptors, but has no impact on immune escape mechanisms[81]. Luan *et al*[82] in an in-silico study, had similarly shown that the N501Y mutation can increase the spike protein's receptor binding affinity with the human ACE2 receptor [82]. The N501Y mutation on RBD may produce an aromatic ring-ring contact and an extra hydrogen bond with ACE2 receptors, increasing binding affinity by tenfold over the wild strain[83]. Moreover, the N501Y mutation decreases the

Table 1 Showing characteristic features of various variants of concerns

VOCs	Transmissibility	Severity	Effect on neutralization by mABs	SGTF	Present status	Vaccine efficacy
Alpha	Increased transmissibility (50%-100%) with R_0 1.75-fold higher compared to original lineage[32]	Increased severity. Hazard of death of 55% (95%CI: 39%–72%) higher than in cases without SGTF after adjustment[33]	No impact on neutralization by mABs, and minimal impact by convalescent and/or post-vaccination sera[18]. E484K and/or various NTD mutations cause a significant fall in neutralization efficacy[39]	Presence	De-escalated	The ChAdOx1 nCoV-19 vaccine showed an efficacy of 70.4% [40]. The first and second dose of BNT162b2 vaccine (Pfizer–BioNTech) reported 48.7% and 93.7% effectiveness, respectively[41]. The reported efficacy of 2-doses of mRNA-1273 vaccine 98.4%[42]
Beta	Increased transmissibility	Increased risk of hospitalization, ICU admission, and mortality in comparison to Alpha and Gamma variants, but less severe disease compared to Delta[44]	45-fold decreased susceptibility to Bamlanivimab-etesevimab therapy. Casirivimab-imdevimab and sotrovimab retained susceptibility[38-40]. Moderate reduction in neutralization by convalescent and post-vaccination sera[45]	Absent	De-escalated	Full vaccination efficacy 73.0% (95%CI: 64.3%–79.5%)[46]
Gamma	1.7 to 2.4-fold higher transmissible than previous (non-P.1) infection [48]. Increased risk of reinfection	1.2 to 1.9 times more likely to result in mortality compared with previous lineages[48]	> 511 fold decreased susceptibility to Bamlanivimab-etesevimab but no change in susceptibility with Casirivimab-imdevimab and Sotrovimab[38-40]. Reduced neutralization to convalescent and post-vaccination sera	Absent	De-escalated	Full vaccination efficacy against Gamma variants 63.0% (95%CI: 47.9%–73.7%)[46]
Delta	40%-60% more transmissible than Alpha variant[49]	Increased severity of the disease[51, 52] and increased risk of hospitalization[52,53].A shorter time interval between disease onset to hospitalization in comparison to the wild-type variant[44]	Neutralization is affected minimally	Absent	De-escalated	Moderate reduction in vaccine efficacy against symptomatic infection but retained efficacy against severe disease and hospitalization[45]. The 2-dose mRNA-1273 vaccine: 86.7% (95%CI: 84.3%-88.7%) efficacy against infection and 97.5% (92.7%-99.2%) efficacy against hospital admission. The 2-doses of BNT162b2 and ChAdOx1 nCoV-19 vaccine 88.0% (95%CI: 85.3%-90.1%) and 67.0% (95%CI: 61.3%-71.8%), respectively[41]
Omicron	Increased risk of transmissibility, reinfection/breakthrough infection	Severity less compared to Delta variant	Reduced or absent neutralization efficacy by vaccines and mABs[56]	SGTF except BA.2 lineage [64]	Few sublineages de-escalated (BA.1, BA.2, BA.3, BA.4, BA.5 *etc*.)	Booster doses are needed to mount a more appropriate immune response against symptomatic or non-symptomatic infections, transmission, and serious manifestations[44]

VOCs: Variants of concerns; SGTF: "*S*" gene target failure; ICU: Intensive care unit; mAbs: Monoclonal antibodies; NTD: N-terminal domain.

polarity of critical residues located in RBD, thereby increasing the affinity between RBD and ACE2 receptors[84,85]. Zhu *et al*[86] reported that a higher number of ACE2 receptors bind with N501Y spikes as compared to N501[86]. Furthermore, using cryo-electron microscopy, the N501Y was placed into a cavity at the binding contact at Y41 of ACE2.This provides a structural basis for the N501Y mutant's higher ACE2 affinity, which is likely related to its greater infectivity. Teruel *et al* [87] in a modeling analysis demonstrated that D614G and N501Y mutations allow the RBD to remain in open conformation for a longer period of time[87]. However, large structural changes in the antibody-binding epitopes do not occur as the N501 is located outside the major neutralizing epitopes on the RBD[88]. Therefore, the N501Y mutation

causes only minimal changes in the sensitivity to neutralizing antibodies. The N501Y mutation co-occurs with several other mutations, such as P681H and deletion of the amino acid at the 69th and 70th residues (Deletion69/Deletion70) on the spike protein. Leung *et al*[89] reported that the N501Y lineage with amino acid deletion Deletion69/Deletion70, detected among the United Kingdom strain, was 75% (70%–80%) more transmissible than the N501 lineage[89]. However, the N501Y mutation does not impact the binding and neutralization of most mAbs[90-95]. Similarly, it rarely shows reduced susceptibility to convalescent plasma[37,92-94].

E484K mutations

The E484K mutation is situated in the RBD and is critical for ACE2 receptor binding and antibody recognition. The E484K mutation has been detected in the B.1.1.7, B.1.351, P.1, and B.1.617.2 variants[53,95]. It involves the replacement of the amino acid glutamic acid (E) with lysine (K) at position 484 of the spike protein. The E484K mutation is an escape mutation, which permits the virus to slip past the body's immunological defenses[95]. Collier *et al*[96] observed that the B.1.1.7 variant carrying the E484K mutation increased the amount of serum antibody needed to prevent infection of cells substantially[96]. The E484K mutations reduce neutralization by antibodies and may cause breakthrough infections[41, 95]. The E484 mutation with amino acid changes to K, Q, or P reduces neutralization by convalescent sera by more than an order of magnitude. Greaney *et al*[97] reported that the E484 mutation with K, Q, or P reduces the neutralization titer of the convalescent plasma collected from the subject on day 32 by 35 to 115-fold[97]. They also found that each of the four discovered mutations (E484A, E484D, E484G, and E484K) conferred resistance to all four convalescent sera tested. The E484 mutation is notable for causing the most significant decreases in neutralization titers. On the other hand, the K444E, G446V, L452R, and F490S mutations escaped three of the four sera tested. The G446V mutation caused approximately a 30-fold decrease in the neutralization titer. By co-incubating the pseudovirus with SARS-CoV-2 spike proteins and mAbs, Liu *et al*[98] demonstrated that that the E484 mutations resulted in considerably lower neutralization efficacy by both mAbs and convalescent sera[98]. Nelson *et al*[99] in a molecular dynamic simulation study, reported that the combination of E484K, K417N, and N501Y mutations resulted in the highest degree of conformational alterations of the RBD domain when bound to ACE2, compared to either E484K or N501Y alone[99]. These mutations favor ACE2 receptor binding. Zahradník *et al*[100] used an *in vitro* evolution model and found that S477N, E484K, and N501Y mutations were among the first to be selected[100]. Moreover, the E484K and N501Y mutations are the tightest binding mutations emerging from the B3 library. Wang *et al*[101] reported that E484K, N501Y individually, or K417N/E484K/N501Y mutations together showed a small but significant reduction in neutralization efficacy with Moderna and Pfizer-BioNTech vaccines[101].

L452R and E484Q mutations

Due to the presence of these two prominent mutations at the same location, it was initially called a “double mutant”. The L452R and E484Q are also the key mutations in the B.1.617.2 variants. The L452R and E484Q double mutants are the two-spike protein RBD mutations and have been detected in 15% to 20% of positive cases in the Maharashtra state of India on March 24, 2021, by the Indian SARSCoV-2 Consortium on Genomics[102]. The L452R and E484Q mutations are responsible for the overall stability of virus-host interaction[103]. They are also responsible for resistance to neutralization by monoclonal and polyclonal antibodies. In the pseudovirus-based study, the L452R mutation caused more cellular entry compared with that of the D614G mutation alone, but it was lower than the N501Y mutation[104]. The L452R mutation raised spike protein expression (0.32 times) and improved binding affinity to ACE2 receptors. It increases the virus's infectivity[84]. The L452R mutation also allows immune escape from human leukocyte antigen (HLA)-restricted cellular immunity[105].

P681R mutation

The furin cleavage site is located at the spike S1/S2 junction. The cleavage of this region is the key to host cell entry. This mutation is responsible for efficient furin cleavage, subsequent internalization, and better transmissibility. A unique feature of the B.1.617.2 variant is the P681R mutation in the spike protein, where proline is substituted by arginine. The P681R mutation is located adjacent to the furin cleavage site[106]. The P681R mutation makes the sequence less acidic and causes furin to function more effectively[51]. Increased furin cleavage will make more spike proteins primed to enter human cells. In the Delta variant, more than 75% of the spikes are primed to infect a human cell, whereas the values in the Alpha variant and original strain were 50% and 10%, respectively[107]. The P681R mutation is highly conserved in the B.1.617.2 variant and is responsible for the higher pathogenicity of the B.1.617.2 variant[108]. P681R mutation in the B.1.617.2 variant enhances SARS-CoV-2 fitness. In an experimental study, Liu *et al*[109] reported that the B.1.617.2 variants outnumbered other variants based on a replication competition assay done on human lung epithelial cells and primary human airway tissues[109]. The mechanism of increased infectivity was explained by the accumulation of the P681R mutation in the B.1.617.2 variant, which causes furin cleavage of the S1/S2 protein, leading to increased infectivity. Moreover, reverting the P681R mutation to wild-type P681 significantly reduced replication.

P681H mutation

The P681H mutation involves the substitution of proline (P) with histidine (H) at position 681. The P681H mutation is also near the S1/S2 furin cleavage site that is responsible for efficient SARS-CoV-2 transmission and infection[85,110]. The P681H mutation may also reduce class 3 antibody recognition[111].

T478K mutation

The T478K mutation is found within the critical receptor binding motif of *S* gene[112]. It alters the virus's affinity for

human cells, increasing viral infectivity. The T478K mutation is a shift in amino acid from polar, uncharged threonine (T) to basic, charged lysine. It may raise the electrostatic potential of spike protein, resulting in a more positive surface in an area that directly contacts ACE2. Furthermore, the longer side chain of lysine is expected to exacerbate the mutant's steric hindrance, perhaps altering the spike/ACE2 interaction[113]. The T478K mutation is frequently co-occurring with three other spike mutations located outside the canonical ACE2 interaction regions, such as D614G (99.83% co-occurrence), P681H, and T732A, with 93.8% and 88.7% co-occurrence with T478K, respectively[114].

N439K mutations

This mutation was identified in March 2020 in Scotland from lineage B.1 on the background of D614G. It has also appeared independently in multiple lineages. As of January 6, 2021, it was reported in 34 countries and was the second most commonly observed RBD mutation worldwide[115]. N439K enhances the binding affinity for the ACE2 receptor and is also responsible for immune evasion. The N439K mutation confers resistance against several neutralizing monoclonal and polyclonal antibodies[116]. The N439K mutation located in the RBD region creates a strong salt bridge with ACE2 receptors, which may enhance the electrostatic complementarity and binding affinity of spike proteins to ACE2[117].

Y453F mutations

The Y453F mutation is located on the RBD and has been detected in human and mink infections. The bidirectional transmission has been reported in the Netherlands[118,119]. Initially, in Denmark, one new lineage was identified and was known as "Cluster 5" and contained mutations in the spike protein[120]. Later on, the mutation was identified as a Y453F mutation located in the RBD domain[121]. The Y453F mutation enhances binding to ACE2. The Y453F mutations involve a tyrosine-to-phenylalanine substitution at amino acid 453 (Y453F). Y453F mutation significantly lowers susceptibility to casirivimab (74-fold), but not to other Food and Drug Administration/Emergency Use Authorisation approved mAbs[122,123]. The Y453F mutation is also found to escape from HLA-restricted cellular immunity[105].

N440K mutations

This mutation was detected in various parts of India in March and April 2021. The N440K mutation is also associated with the P323L substitution in the *RdRP* gene. The N440K variant can generate significantly higher viral loads within a short period, leading to its rapid spread. This variant has shown localized spread in the following four states: Karnataka, Maharashtra, Telangana, and Chhattisgarh. They together contributed to about 50% of these samples submitted for analysis[124]. The N440K mutation has also been reported to cause reinfection[125]. The frequency of the N440K variant was 2.1% in India and was particularly high in the state of Andhra Pradesh (33.8% of 272 genomes)[126]. The N440K variant is responsible for immune escape as it has shown resistance to class 3 mAbs and an enhanced binding affinity to the human ACE2 receptor[127].

AMINO-TERMINAL DOMAIN OR NTD MUTATIONS

NTD mutations in spike protein are often the neglected area in the SARS-CoV-2 genomic study. However, NTD mutations have been reported among the B.1.1.7 and B.1.351 lineages[128]. A significant transmission of a six-nt deletion in the *S* gene has been reported by Gupta *et al*[128] leading to a loss of two amino acids: H69 and V70[128].

Here we report recurrent emergence and significant onward transmission of a six-nt deletion in the *S* gene, which results in loss of two amino acids: H69 and V70. The H69/V70 variant also co-occurs with N501Y, N439K, and Y453F mutations on the RBD. The H69/V70 deletion increases infectivity twofold, and the effect on viral fitness is independent of the RBD changes. The H69/V70 mutations may also boost SARS-CoV-2's ability to generate new variants, such as vaccine escape variants[129].

K417N/T mutations

The K417N/T mutation has been reported in the B.1.351 (as K417N), P.1 (as K417T), and B.1.1.529 variants (as K417N). Interestingly, the K417N/T mutation usually occurs in presence of other RBM mutations as these mutations may decrease the binding to ACE2 receptors[84,115]. The K417N/T mutation may cause immune evasion as well. The K417N mutation confers reduced susceptibility to etesevimab[130] and casirivimab[92] but retains susceptibility to bamlanivimab, imdevimab, and sotrovimab[124]. It also retains susceptibility to convalescent plasma or sera from patients vaccinated with the mRNA vaccine[92,123]. The K417N, E484K, or N501Y mutations showed a reduced or abolished neutralization by 14 of the 17 most potent mAbs tested[101]. Li *et al*[131] in a pseudovirus model, showed that the K417N mutation increases viral sensitivity to neutralization. Normally, the K417 variant allows a closed conformation, leading to reduced binding to ACE2 and reduced access to neutralization antibodies. The K417N mutation helps in an open conformation, resulting in the exposure of more epitopes to neutralizing antibodies and subsequently increased virus neutralization. Table 2 shows the five VOCs and their mutations.

CONCLUSION

The emergence of SARS-CoV-2 variants is an important phenomenon in the natural history of SARS-CoV-2 infection

Table 2 Showing the five variants of concerns and their mutations

WHO label	Pango lineage	GISAID clade	Nextstrain clade	Spike protein substitutions	First detected	WHO date of designation
Alpha	B.1.1.7	GRY	201(V1)	Deletion 69-70, Deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	United Kingdom	18th December 2020
Beta	B.1.351	GH/501Y.V2	20H(V2)	D80A, D215G, DeletionL242, DeletionA243, DeletionL244, K417N, E484K, N501Y, D614G, A701V	South Africa	18th December 2020
Delta	B.1.617.2	G/478K.V1	21A	T19R, T95I, G142D, Deletion156, Deletion157, R158G, L452R, T478K, D614G, P681R and D950N	India	VOI: 4th April, 2021 VOC: 11th May, 2021
Gamma	P.1	GR/501Y.V3	20J(V3)	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I	Brazil	11th January 2021
Omicron	B.1.1.529 lineage	GR/484A	21K	A76V, T95I, Y145del, G339D, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F, L212I, S371L, S373P, S375F, K417N. ORF1a: K856R, ORF1a: L2084I, ORF1a: A2710T, ORF1a: T3255I, ORF1a: P3395H, ORF1a: I3758V, ORF1b: P314L, ORF1b: I1566V, and ORF9b: P10S	Botswana and South Africa	26th November 2021

WHO: World health organization; GISAID: Global initiative on sharing all influenza data; VOI: Variants of interest; VOC: Variants of concern.

because it poses a considerable public health risk. Currently, we have five VOCs. These variants are more transmissible than the Wuhan strain. Various mutations identified in these VOCs are located on the spike protein, especially in the RBD. These mutations influence virus-host cell interaction, binding affinity, furin cleavage, and neutralizing efficacy by antibodies and vaccines. The most recent VOC detected is the omicron variant; however, this will not be the last variant we encounter. We will also see newer variants in the future, too. Characterization of the genomic character of the VOCs will help in identifying newer mutations quickly and in exploring phenotypic effects on the virus. In this article, we looked at the characteristics of the five VOCs, as well as the associated mutations, and how they affect SARS-CoV-2 virus's infectivity, transmissibility, and immune evasion. The best way to prevent the development of new variants is to vaccinate as many people as possible, closely adhere to infection prevention and control measures, and eliminate vaccine inequalities that limit future human transmission and acquisition.

FOOTNOTES

Author contributions: Sarkar M conceived and designed the experiment, made critical revisions, and approved the final version; Madabhavi I and Sarkar M analyzed the previous studies and research and wrote the first draft of the manuscript, contributed to the writing of the manuscript, jointly developed the structure and arguments for the paper. All authors reviewed and approved the final manuscript.

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Country/Territory of origin: India

ORCID number: Irappa Madabhavi 0000-0002-8543-2413.

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MINIREVIEWS

Stent A pancreaticojejunostomy after pancreatoduodenectomy: Is it always necessary?

Dimitrios Symeonidis, Dimitris Zacharoulis, Georgios Tzovaras, Labrini Kissa, Athina A Samara, Eleana Petsa, Konstantinos Tepetes

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Dimitrios Symeonidis, Dimitris Zacharoulis, Georgios Tzovaras, Labrini Kissa, Athina A Samara, Eleana Petsa, Department of Surgery, University Hospital of Larissa, Larissa 41110, Greece

Konstantinos Tepetes, Department of General Surgery, University Hospital of Larissa, Larissa 41110, Greece

Corresponding author: Athina A Samara, MD, MSc, Surgeon, Department of Surgery, University Hospital of Larissa, Mezourlo Hill, Larissa 41110, Greece. at.samara93@gmail.com

Abstract

The establishment of a postoperative pancreatic fistula (POPF) is considered the most common and, concomitantly, the most serious complication associated with pancreaticoduodenectomy (PD). The search for either technical modifications of the operative technique or pharmaceutical interventions that could possibly aid in decreasing the incidence of this often-devastating complication appears justified. The stenting of the pancreatic duct, with the use of either internal or external stents, has been evaluated in this direction. In theory, it is an approach that could eliminate many pathophysiological factors responsible for the occurrence of a POPF. The purpose of the present study was to review the current data regarding the role of pancreatic duct stenting on the incidence of POPF, after PD, by using PubMed and Reference Citation Analysis. In general, previous studies seem to highlight the superiority of external stents over their internal counterparts in regard to the incidence of POPF; this is at the cost, however, of increased morbidity associated mainly with the stent removal. Certainly, the use of an internal stent is a less invasive approach with acceptable results and is definitely deprived of the drawbacks arising through the complete diversion of pancreatic juice from the gastrointestinal tract. Bearing in mind the scarcity of high-quality data on the subject, an approach of reserving stent placement for the high-risk for POPF patients and individualizing the selection between the use of an internal or an external stent according to the distinct characteristics of each individual case scenario appears appropriate.

Key Words: Pancreaticoduodenectomy; Postoperative pancreatic fistula; Pancreatic stent; Pancreaticojejunostomy

Core Tip: A postoperative pancreatic fistula (POPF) is considered the most common and the most serious complication associated with pancreaticoduodenectomy. Reserving stent placement for the high-risk for POPF patients and individualizing the selection between the use of an internal or an external stent according to the distinct characteristics of each individual case scenario appears appropriate.

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INTRODUCTION

Pancreaticoduodenectomy (PD) is the procedure of choice for the surgical treatment of both benign and malignant lesions of the pancreatic head and the periampullary region. In recent years, significant progress has been made in regard to the outcomes of this highly demanding operation. Mortality rates of less than 5% have been reported among specialized centers worldwide with high hospital volume considered, at least in part, responsible for this impressive outcome[1,2]. However, despite this favorable development, morbidity remains a major issue after any kind of pancreatic surgery. The establishment of a postoperative pancreatic fistula (POPF) is considered the most common and, concomitantly, the most serious complication associated with PD, with incidence varying in the literature between 5 and 40%, depending on the definition used[3].

In 2005, the International Study Group of Pancreatic Fistula, aiming to overcome problems associated with the absence of a universally adopted definition, developed a definition and grading scheme of POPF[4]. According to this definition, a pancreatic fistula is defined as a drain output of any measurable volume of fluid starting from the third postoperative day with amylase content greater than 3 times the serum amylase activity. Subsequently, 3 different grades of POPF (grades A, B, and C) were defined based on the clinical impact of POPF on the patients' clinical course. In 2016, the International Study Group of Pancreatic Fistula reconvened as the International Study Group of Pancreatic Surgery (ISGPS) to review the recent literature and update the 2005 definition and grading system of POPF. In the updated definition, the clinically relevant POPF is now redefined as a drain output of any measurable volume of fluid with an amylase level of more than 3 times the upper limit of the institutional normal serum amylase activity, associated with a clinically relevant condition related directly to the POPF. Therefore, the former grade A POPF is now called a "biochemical leak." A grade B POPF requires the modification of the postoperative management while the drains are either left in place for more than 3 wk or are repositioned with the use of endoscopic or minimally invasive percutaneous procedures. Finally, patients with Grade C POPF require reoperation or have signs of organ failure[5].

In general, a pancreaticojejunostomy—that is, an anastomosis between the pancreatic stump and a jejunal loop—has been established as the standard and most commonly applied method of reconstruction following PD[6]. A POPF represents the clinical manifestation of a failing and inefficient anastomosis. The quest for either technical modifications or pharmaceutical interventions that could possibly aid in decreasing the incidence of this often-devastating complication appears justified. Performing a pancreaticogastrostomy over a pancreaticojejunostomy has been tested in this direction, but literature data in regard to the efficiency of the approach are contradictory[7]. Furthermore, the former seems to be associated with an increased incidence of post-pancreatectomy haemorrhage[7]. Similarly, the pancreatic duct occlusion or the use of fibrin glue to reinforce the anastomosis did not seem to have the desired results[8-10]. Apart from the various proposed technical modifications of the operative technique, pharmaceutical agents have been tested as well. In theory, somatostatin analogues could limit the incidence of POPF by decreasing exocrine pancreatic secretion. However, a recent meta-analysis demonstrated that the administration of somatostatin analogues such as octreotide did not affect the incidence of POPF and clinically relevant POPF after PD[11]. The stenting of the pancreatic duct, with the use of either internal or external stents, has also been evaluated because, at least in theory, it is an approach that could eliminate many pathophysiological factors responsible for the occurrence of a POPF. The purpose of the present review was to assess the role of pancreatic duct stenting on the incidence of POPF, after PD, by reviewing the relevant literature.

RISK FACTORS FOR POPF

The determination of risk factors for the development of a POPF has been a field of constant research. Ideally, a process of objectifying and easily reproducing the risk assessment could more accurately target possible interventions or deviations from the standard practice selectively to the high-risk patient groups. Therefore, the possible benefits from every intervention that could act protectively, against the development of a POPF, could be augmented. In 2013, Callery *et al*[12] proposed and validated a clinical risk score, that is, the fistula risk score that could objectively quantify the risk for POPF. The authors assessed and calibrated 4 distinct and widely acknowledged risk factors for POPF after PD, namely the small diameter of the pancreatic duct, the "soft" texture of the pancreatic parenchyma, the presence of high-risk pathology, and the excessive intraoperative blood loss. The combination of these factors, which correlated strongly with the occurrence of a POPF, afforded a 10-point fistula risk score of high predictive value. In general, patients with scores of 0 points, within

the validation cohort, never developed a POPF, whereas fistulas occurred in all patients with a score of more than 9[12].

An alternative fistula risk score was proposed by Mungroop *et al*[13] in 2019 in an attempt to eliminate blood loss as a predictor for POPF. The blood loss factor had been only weakly correlated with the end point of POPF, and the authors aimed to test the hypothesis of developing a risk score taking into account only 3 predictors of POPF development, namely the pancreatic texture, the pancreatic duct diameter, and the body mass index. The alternative fistula risk score was externally validated in 2 independent databases (University Hospital of Verona and University Hospital of Pennsylvania), using both 2005 and 2016 ISGPS definitions, and its predictive value was adequately documented[13]. However, as the penetration of minimally invasive surgery in the field of pancreatic surgery was constantly increasing, the need to validate and optimize the alternative fistula risk score for patients undergoing minimally invasive PD also became mandatory. The updated alternative fistula risk score, which included male sex as a risk factor for POPF development, was the result of a validation study performed in a pan-European cohort of 952 consecutive patients undergoing minimally invasive PD in 26 centers from 7 countries[14].

PROS AND CONS OF PANCREATIC DUCT STENTING

The development of a POPF represents a major source of morbidity and even mortality after a PD[1-3]. The direct and indirect consequences of a POPF can significantly complicate the patient's postoperative course. An intra-abdominal hemorrhage, an abscess formation, delayed gastric emptying, or the significant delay of bowel function in the postoperative period represent only some of the possible indirect dismal effects of a POPF. From the pathophysiological viewpoint, 3 important factors could be postulated in the aetiology of a pancreatic fistula: First, the poor surgical technique resulting in a not-watertight anastomosis that is, in turn, highly susceptible to leaks; second, the increased intraluminal pressure within the jejunal loop that is purposed to contain and propel the pancreatic juice; third, the destructive effect of the activated pancreatic enzymes on an immature anastomosis that can magnify clinically insignificant leaks.

In general, 2 stent types sized between 5 and 8 Fr, depending on the pancreatic duct size, have been tested in regard to their efficiency in reducing the incidence of POPF after PD, that is, internal and external stents. An external stent is a plastic catheter inserted into the main pancreatic duct and is purposed to drain the pancreatic juice originating from the main pancreatic duct directly outside the abdominal cavity. In contrast, an internal stent is similarly a plastic catheter, though significantly smaller in length than an external stent, purposed to direct the pancreatic juice into the intestinal lumen[15]. From the technical viewpoint, the use of stents, either internal or external, during the maturing process of a pancreaticojejunostomy can efficiently prevent the inadvertent iatrogenic occlusion of the main pancreatic duct, irrespective of the adopted technique[15].

In 1999, Roder and Stein set the scene for the introduction and the establishment of pancreatic stents in pancreatic surgery by reporting an impressive decrease in POPF rate, from 29.3% to 6.8%, with the use of external stents[15]. In general, the rationale for using an external stent is the increased short-term safety and, up to a point, guaranteed clinical stability in the immediate postoperative period. In support of this, one of the most decisive interventions in the therapeutic setting—that is, after a clinically significant POPF has already been established—is the external drainage, *via* a catheter, of the pancreatic juice[16]. Thus, proactively thinking, the use of an external stent during the index operation could effectively prevent the accumulation of pancreatic juice within the jejunal loop, which is anastomosed with the pancreatic stump, and subsequently disrupt the pathophysiologic cascade of events that eventually could result in the occurrence of a POPF[17]. The issue of the increased intraluminal pressure, as one of the causes of POPF, which is further magnified in the immediate postoperative period due to the decreased gastrointestinal motility, seems to be adequately addressed by the external stenting approach[18]. Furthermore, the complete diversion of pancreatic juice prevents the activation of the pancreatic enzymes by the enzyme, enterokinase, within the jejunal lumen[19]. In theory, protecting a healing anastomosis from the corrosive effect of highly active pancreatic enzymes could increase the likelihood of an uneventful maturing of the anastomosis.

However, there are also drawbacks associated with the approach of externally stenting the pancreaticojejunostomy. Digestive enzymes of significant physiological value are diverted and, ultimately, deprived from the gastrointestinal tract. Impairments on gastrointestinal tract motility and on the absorption of valuable, during the immediate postoperative course, nutrients should be anticipated with mainly unknown clinical implementations. In addition, the stent-related complications are not negligible. Drainage tube discomfort, displacement, and shedding or clogging resulting in peritonitis can all occur and significantly raise morbidity and mortality rates[20-22]. Finally, mechanical injury of the pancreatic duct, at the level of the anastomosis, may likely occur during stent removal, resulting in pancreatitis or obstruction of the pancreatic duct[23,24]. Ohwada *et al*[25] reported 2 cases (5.4%) of local peritonitis associated with the removal of external stents after PD.

That said, the use of an internal stent should be considered a less radical approach detached by the majority of the limitations associated with the use of external stents. Internal stents could in theory be associated with better long-term outcomes because they are associated with decreased risk of pancreatic duct dilation and endocrine dysfunction compared to external stents[26]. Guiding the pancreatic juice toward the appropriate direction rather than externally diverting it and aiding in performing a technically optimal anastomosis in cases of pancreatic ducts of small diameter are the rationale behind the use of an internal stent. Irrespective of their effectiveness in reducing the incidence of POPF, an internal stent does not have to be removed, and it is associated with fewer fluid losses, water–electrolyte imbalance, impaired gastrointestinal function, internal environment disturbance, malnutrition, and other risks[26]. Preoperative nutrition status plays an important role in predicting the risk of POPF, and several scores have been proposed so far[27].

TO STENT OR NOT TO STENT A PD

The goal behind the use of stents, inserted within the main pancreatic duct during the reconstruction process following PD, is to reduce the incidence of POPF. Several clinical controlled trials and 7 randomized controlled trials (RCTs) have been conducted to assess the impact of stents on this matter, although with conflicting results[21,26-32]. Recently, 2 meta–analyses were published with the aim of summarizing the currently available evidence.

In the meta-analysis by Jiang *et al*[33], 4 RCTs and 6 non-randomized trials with a total of 2101 patients were included. According to the results, the use of an external stent yielded superior results over the use of an internal stent, in terms of POPF grade C occurrence. However, the use of stent, irrespective of the type, did not reduce the rate of POPF grade B in all studies. The authors concluded that compared with internal stents, the use of external stent might be associated with a lower rate of pancreatic fistula grade C but underlined the need for more high-quality evidence to further explore the safety and efficacy of pancreatic duct external stents[33]. In 2022, Guo *et al*[34] published another meta-analysis including all the available RCTs and a total of 847 patients with more or less respective results. The authors reported no statistically significant difference between the stent group and non-stent group in the incidence of POPF, in-hospital mortality, reoperation, delayed gastric emptying rate, and wound infection. However, the subgroup analyses revealed that the use of an external stent significantly reduced the incidence of POPF.

DISCUSSION

The development of a clinically relevant POPF—that is, grade B or C, according to the most recent ISGPS definition—remains the most challenging complication after PD[5]. Practically, a pancreatic fistula represents the clinical manifestation of a failing pancreatico–enteric anastomosis. Multiple techniques and, in general, various strategies such as pancreatic duct stenting or the administration of somatostatin analogues have been tested in the direction of reducing the incidence of this troubling complication. However, until today, no single method has proved absolutely efficient. In 2017, the ISGPS published a position statement in regard to the optimal method of reestablishing the continuity of the pancreatic stump with the rest of the gastrointestinal tract after PD. According to this statement, there is no specific technique—that is, a pancreaticogastrostomy or a pancreaticojejunostomy—that can guarantee the complete elimination of the incidence of a clinically relevant POPF. Specifically, in regard to the suggested role of pancreatic stents during PD, the authors underlined the scarcity of high-quality data and the need for further research in the field[35].

In practice, there is low risk for the development of POPF patients, and things are relatively straightforward. The incidence of POPF is limited, and there is no innate need for utilizing adjuncts to improve the outcome. However, problems arise when a high risk is present for a POPF patient. The several published fistula risk scores are particularly aimed at accurately defining this high-risk patient group. Factors such as the soft texture of the pancreatic parenchyma, the small diameter of the main pancreatic duct, male gender, as well as certain anthropometric variables can predict an increased likelihood of a technically difficult pancreaticojejunostomy with high associated failure rate and, subsequently, POPF development[12-14]. In this setting, POPF rates of even more than 30% could be anticipated[14]. Studies regarding the use of fibrin sealants during pancreatic surgery to reduce POPF have been published with controversial results. In a Cochrane review in 2020, the researchers concluded that based on the then-current available evidence, fibrin sealants may have little or no effect on postoperative pancreatic fistula in people undergoing distal pancreatectomy[36].

An approach of utilizing adjuncts such as stents during PD, in these high-risk patients, appears justified[37]. Irrespective of the stent type that is highlighted in the literature as superior, there are reports that do underline the benefits of the approach. Jiang *et al*[38] analyzed a cohort of 172 patients at high risk for POPF and reported that the use of an external stent could, indeed, reduce the incidence of clinically relevant POPF in patients with a fistula risk score ≥ 4. Conversely, Kawai *et al*[39] highlighted the superiority of internal stents based on the results of a multicenter large cohort study using propensity score-matched analysis comparing internal and external stents for pancreatojejunostomy during PD. According to the results, clinically relevant POPF occurred in more patients in the external stents group than in patients in the internal stents group (28.7% *vs* 12.9%, $P < 0.001$). Particularly for the high-risk group (soft pancreas and no dilatation of the pancreatic duct), the rate of clinically relevant POPF in the internal and external stents groups was 18.8% and 35.4% respectively. The authors concluded that internal stents are safer than external stents for PD.

The task of summing up and analyzing all these controversial and confusing data is rather difficult (Figure 1). Drawing definite conclusions based on the existing evidence appears inappropriate. However, some factors can certainly be underlined. Performing a pancreaticojejunostomy over a stent, especially when conditions that do not guarantee a favorable outcome are present, can create the conditions for a safer and technically sound anastomosis. The inadvertent occlusion of a small (in diameter) pancreatic duct that can be prevented by the use of a stent could compromise the operative outcomes. In practice, when a small duct is the case, performing the pancreaticojejunostomy over a pancreatic stent has become commonplace in the majority of specialized centers worldwide[40,41].

CONCLUSION

In conclusion, literature reports seem to highlight, in their majority, the superiority of external stents over their internal counterparts in regard to the incidence of POPF, albeit at the cost of increased morbidity associated mainly with the stent removal. Certainly, the use of an internal stent is a less invasive approach with acceptable results and definitely lacking the drawbacks arising through the complete diversion of pancreatic juice from the gastrointestinal tract. Bearing in mind

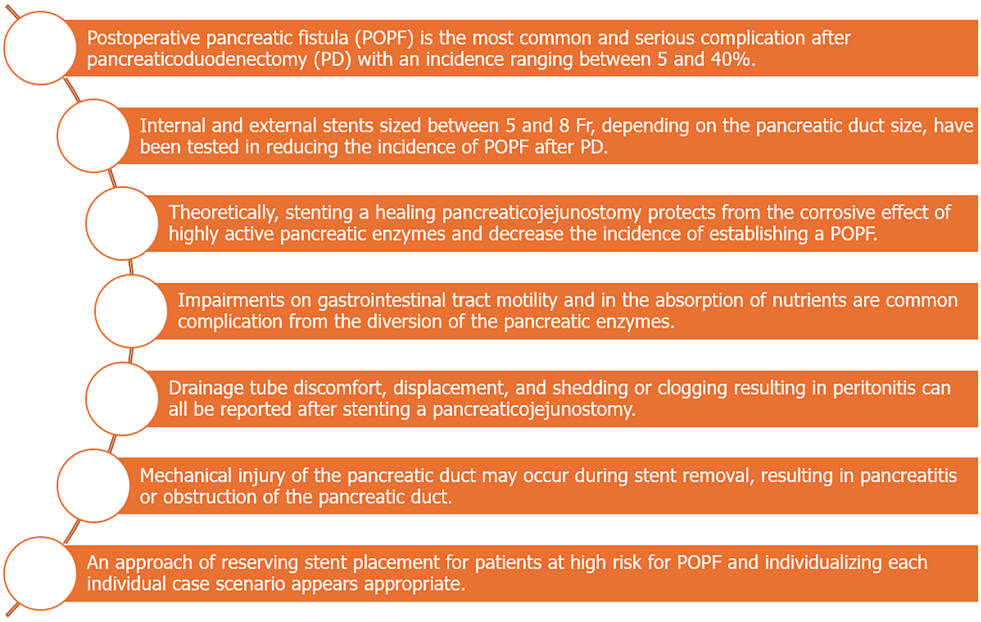

Figure 1 Key-points of stenting a pancreaticojejunostomy after pancreatoduodenectomy.

the scarcity of high-quality data on the subject, an approach of reserving stent placement for patients at high risk for POPF and individualizing the selection between the use of an internal or an external stent according to the distinct characteristics of each individual case scenario appears appropriate.

FOOTNOTES

Author contributions: Symeonidis D, Zacharoulis D, and Samara AA contributed to study conception and design; Kissa L and Petsa E contributed to acquisition of data; Tzovaras G and Samara AA contributed to analysis and interpretation of data; Symeonidis D, Zacharoulis D, and Samara AA contributed to drafting of manuscript; Tzovaras G, Kissa L, Petsa E, and Tepetes K contributed to critical revision; All authors have approved the final version of the present manuscript.

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Country/Territory of origin: Greece

ORCID number: Dimitris Zacharoulis 0000-0003-0107-3976; Georgios Tzovaras 0000-0001-5322-8269; Athina A Samara 0000-0002-6177-7281; Konstantinos Tepetes 0000-0001-9435-6849.

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MINIREVIEWS

Pain in chronic pancreatitis: What can we do today?

Margherita Binetti, Valeria Tonini

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Margherita Binetti, Valeria Tonini, Department of Medical and Surgical Science, University of Bologna, Alma mater Studiorum, Bologna 40138, Italy

Corresponding author: Valeria Tonini, MD, Academic Research, Doctor, Researcher, Surgeon, Surgical Oncologist, Department of Medical and Surgical Science, University of Bologna, Via Massarenti 9, Bologna 40138, Italy. valeria.tonini@unibo.it

Abstract

The aim of this study is to illustrate the complexity of pain management in chronic pancreatitis (CP). In this context, pain represents the most common and debilitating symptom, and it deeply affects patient's quality of life. Multiple rating scales (unidimensional, bidimensional and multidimensional) have been proposed to quantify CP pain. However, it represents the result of complex mechanisms, involving genetic, neuropathic and neurogenic factors. Considering all these aspects, the treatment should be discussed in a multidisciplinary setting and it should be approached in a stepwise manner. First, a lifestyle change is recommended and nonsteroidal anti-inflammatory drugs represent the gold standard among medical treatments for CP patients. The second step, after medical approach, is endoscopic therapy, especially for complicated CP. In case of failure, tailored surgery represents the third step and decompressive or resection procedures can be chosen. In conclusion, CP pain's management is challenging considering all these complex aspects and the lack of international protocols.

Key Words: Chronic pancreatitis; Pain; Multifactorial mechanism; Stepwise approach; Endoscopic treatment; Early surgery

Core Tip: The aim of this review is to analyse and discuss treatment options in chronic pancreatitis management. Lifestyle change represents the starting point in uncomplicated chronic pancreatitis (CP). Medical treatment should be the first considered in a stepwise approach. The use of nonsteroidal anti-inflammatory drugs is the gold standard, but opioids, antioxidants, neuromodulators have important roles as well. Endoscopic retrograde cholangiopancreatography, extracorporeal shock wave lithotripsy with or without endoscopy, sphincterotomy with stent placement or transgastric drainage can be chosen in complicated CP patients with obstructions or pseudocysts. A decompressive or resection operation can be chosen in surgical treatment. In conclusion, CP pain management is an ongoing challenge because of lack of international consensus on protocols. Nowadays, a tailored step-up treatment discussed in a multidisciplinary setting is considered the best approach.

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INTRODUCTION

Chronic pancreatitis (CP) is a progressive pancreatic disorder characterized by inflammation and fibrosis. The incidence and prevalence of CP remain low. The incidence is estimated about 4-12 per 100000 persons/year; while the prevalence is about 37-42 per 100000 persons/year. Abdominal pain represents its most disabling manifestation, and its prevalence in CP is about 80% of patients; painless pancreatitis only presents in 10%-20% of cases. In painless CP steatorrhea, malabsorption and endocrine dysfunction often develop[1].

In addition, acute and chronic pancreatitis represents a leading cause of hospital admissions[1]. Both genetic and environmental factors contribute to CP[2]. CP risk factors are summarised by the TIGAR-O acronym: *T* = *Toxic* (alcohol abuse, tobacco smoking, medications or toxins), *I* = *Idiopathic* (not associated with any known gene), *G* = *Gene Mutation* (complex genetics or modifying genes, ex. PRSS1, CFTR, SPINK1), *A* = *Autoimmune* (steroid responsive chronic pancreatitis), *R* = *Recurrent* (CP due to vascular diseases and post-irradiation damage), *O* = *Obstructive* (CP associated with pancreas divisum, Sphincter of Oddi disorder and duct obstruction)[3] and the M-ANNHEIM acronym includes: Alcohol and nicotine consumption, nutritional and hereditary factors, Efferent duct, Immunological, Miscellaneous and rare metabolic factors[4].

CP generally occurs together with mid-epigastric abdominal pain associated with nausea and vomiting[5]. In fact, abdominal pain represents the most frequent and debilitating symptom of chronic pancreatitis[6], and up to the 80% of patients with CP present recurrent episodes[2]. It is usually described starting in the epigastric zone with radiation to the back but may present variability[7]. The possible complete resolution of pain after the ongoing loss of pancreatic exocrine function remains a controversial topic[5]. CP presence could be associated to new onset diabetes (20%), steatorrhea (19%) and weight loss (16%) in painless patients[8]. CP patients can experience increased pain after eating, potentially leading to poor nutrition intake[9]. According to a recent review by Lukic *et al*[10] "chronic" abdominal pain persists for more than 3 mo.

Pain in CP has both somatic and visceral components. The afferent nerves of viscera terminate at various levels of the spinal cord, leading to a diffuse pain feeling. Part of the nerve projections involve sympathetic fibres, leading to nausea, diarrhoea and early satiety[11]. Considering CP clinical presentation, pain represents the most debilitating factor[3]. It has a great effect on quality of life (QoL). Pain severity can present as mild-moderate (18%) or severe (67%), and pain frequency can be intermittent (32%) or constant (53%)[2]. The aim of this study is to illustrate the complexity of pain management in CP. The underlying mechanisms of pain have been analysed, both in neuropathic and nociceptive components. The genetic role has been also described. After CP pain diagnosis, some unidimensional, bidimensional or multidimensional scales may be used to quantify the chronic pain. International guidelines have not been published yet. However, medical treatment is recommended as a first approach. In case of failure, endoscopic options can be tested. Surgical options should be chosen only in case of medical and endoscopic failure.

DIAGNOSIS

The diagnosis of CP remains a clinical challenge[12]. According to the United European Gastroenterology evidence-based Guidelines about CP diagnosis, endoscopic ultrasonography (US) recruits the highest possible number of patients, while endoscopic retrograde cholangiopancreatography (ERCP) and transabdominal US have the highest and lowest sensitivity, respectively[12].

Different scales can be used for pain assessment. The Numerical Pain Rating Scale is a one-dimensional rating scale and it is widely recommended, but multidimensional ones such as the Brief Pain Inventory and the McGill Pain Questionnaire are preferred[12]. General pain assessment tools can differentiate between[13]: Unidimensional tools: Pain visual analogue scale (VAS), pain numerical rating scale, pain intensity categories (mild, moderate, severe), pain improvement/relief categories, pain pattern (constant/intermitted), postprandial pain (yes/no or intensity); Bidimensional tools: Daily pain duration median pain VAS, number of days with pain median pain VAS, number of hours of pain median pain VAS, degree of frequency median pain VAS, pain frequency pain severity; Multidimensional tools:

McGill Pain Questionnaire (full and short-form), PainDetect Questionnaire, pain score (intensity, frequency and consequences of pain). Some specific pain assessment tools in CP are reported: Izbicki pain score, Ammann (Type A&B), Type A-E, Group 1e3 pain patterns, QLQ-PAN28[13].

PAIN MECHANISMS

The mechanism of CP abdominal pain is complex. Although pancreatic damage represents a fundamental component, it also involves both nociceptive function and central pain perception[14,15].

Regarding pancreatic damage, acinar cell injury and pancreatic duct obstruction cause parenchymal ischemia, which is the base of abdominal pain in CP. This local ischemia induces inflammation that causes nociceptive stimulation of peripancreatic nerves. Repetitive stimulations can lead to permanent changes in spinal cord and cerebral cortex[7].

Nociceptive pain occurs after primary afferent neuron activation due to chemical or mechanical stimuli[16,17]. The exact nature of factors that actually activate intrapancreatic nociceptors is still unknown[18].

Neuropathy is an important component of CP pain. The continuous sensitization of central nociceptive receptors may result in a self-perpetuating pain state, which is independent from peripheral input[19]. Intrapancreatic nerves both increase in size (neural hypertrophy) and in number (neural invasion)[20].

In 2010, some authors analysed cortical reorganization in CP patients. They showed prolonged latencies of evoked potentials in the frontal region and in insular dipole localization. These findings showed that prolonged pain in CP patients leads to central reorganization[21]. The constant stimulation of afferent pathways leads to neuroplastic changes in the central nervous system (CNS) with overactivity of pain-related structures in a chronic activation setting. Various CNS areas are involved, such as the medial thalamus, the somatosensory cortex, the parietoinsular cortical regions and limbic areas[22].

Some biochemical studies have analysed specific molecules implicated in CP pain mechanisms. According to some authors, pancreatic nociceptor involvement with an increased excitability seems to be related to K+ current downregulation. TRPV1, nerve growth factor and protease activated receptor 2 seem to be involved[23]. Biochemical and histopathological characteristics in CP patients are similar to those observed in patients with other nerve fibre lesions[24]. Compared to healthy controls, CP patients also have increased glutamate/creatine (glu/cre) levels in the anterior cingulate cortex, while they have reduced N-acetylaspartete/creatine (NAA/cre) levels[25]. These mechanisms have been revealed by cerebral spectroscopy.

Chronic pancreatitis' genetic profile has also been analysed. It plays an important role in pain perception and tolerance. Serum levels of transforming growth factor beta 1 seem to be higher in patients with nociceptive pain, while GP130 seems to be marker for neuropathic pain[26]. Some studies also suggest a role for neuromodulator drugs in the treatment of pain based on genetic susceptibility[27].

Over the years, many different theories have been proposed about the origin of pain in CP. It represents a multifactorial process. "Pancreatic duct hypertension" is considered one of the most accredited theories[28]. A direct relationship between pain and duct hypertension was first described by White *et al*[29]. It has been reproduced by infusing saline infusion with ductal pressure exceeding 25 mmHg.

In addition to histological changes, there are also CP-related functional changes[30], including maldigestion, diarrhea, weight loss and diabetes mellitus following islet-cell dysfunction[28].

CURRENT GUIDELINES

The official guidelines for CP pain treatment have been prepared following CP clinical and diagnostic criteria revisions. The first guideline was created in 2009[31] and the second one in 2015[32]. The third edition was published in 2022, "Evidence-based clinical practice guidelines for chronic pancreatitis"[33], after the redefinition of CP as a pathogenic fibro-inflammatory syndrome.

The guidelines for pain management of pain In chronic pancreatitis (2017) contain recommendations from the Working Group for the International Consensus Guidelines for Chronic Pancreatitis in collaboration with the International Association of Pancreatology, American Pancreatic Association, Japan Pancreas Society and European Pancreatic Club[6].

The European Society for Gastrointestinal Endoscopy Guidelines recommend, in case of obstruction of pancreas head or body, endoscopic therapy with Extracorporeal shock wave lithotripsy with or without endoscopy (ESWL) as first treatment, followed by re-evaluation 6-8 wk later[34].

However, absolute indications about CP treatment are missing due to the lack of standardized protocols. International guidelines recognise a lack of international consensus about diagnostic tools and validated assessment in CP pain management.

PAIN MANAGEMENT IN CHRONIC PANCREATITIS

Abdominal pain is a complex symptom and requires a tailored treatment[7]. Traditional pain management starts with lifestyle changes, such as cessation of both smoking and alcohol consumption[5,35]. According to 2017 guidelines abstinence from smoking has a weak recommendation, while abstinence from alcohol has a moderate recommendation

[6]. Correct treatment of CP pain involves either anatomic and neurologic contribution to pain[7].

The World Health Organization recommends a stepwise approach[4]. To examinate CP pain management, it is useful to differentiate: CP Simple abdominal pain/back pain management; Complicated CP management: pancreatic pseudocyst, internal pancreatic fistula, biliary stenosis (Figure 1).

PAIN TREATMENT IN CHRONIC PANCREATITIS

Abdominal or back pain are the most frequent presentation in uncomplicated CP.

Medical

Medical therapies are recommended for patients without pancreatic duct obstruction, with a lower severity of pain[7] according to a "pain relief ladder" principle, as proposed by the World Health Organization[19].

A stepwise CP pain management approach begins with acetaminophen and non-steroidal anti-inflammatory drugs, followed by low potency and longer acting opioids[4].

Medical therapies

Acetaminophen: For many authors it is the first choice[36]. However, according to other authors, Paracetamol is safe but does not result in satisfactory pain relief[37].

Nonsteroidal anti-inflammatory drugs: According to the majority of authors, nonsteroidal anti-inflammatory drugs (NSAIDs) represent the first choice for analgesia in CP pain control. Only few studies evaluated the efficacy of various analgesics[38].

Opioids: Opioid analgesics are additive therapy in case of persistent or increasing pain. In this context, opioid use disorder is a risk. The careful selection of CP patients who would benefit from opioid therapy and predicting the risk of potential misuse should be applied[36]. According to Ratnayake *et al*[39], spinal cord stimulation is effective on reducing CP pain and has a potential effective role in reducing opioid use.

Antioxidants: Many antioxidants including vitamin A, C, E, selenium and methionine have been proven. The goal of antioxidant use is to decrease 'ischemia-induced inflammation', which could represent a peri-pancreatic nerve stimulus [4]. A sufficient dose of antioxidants should be recommended. However, according to some other authors, antioxidants are not related to better CP pain control[6,40]. A recent study concludes that a combination of antioxidants and Pregabalin significantly reduces CP pain[41].

Neuromodulators: Pregabalin, gabapentin, tricyclic antidepressants: Pregabalin was shown to reduce daily pain scores compared to placebo in a randomized study[7]. Considering 64 enrolled total patients, 36% of Pregabalin-treated patients against 24% Placebo-treated patients reported pain relief[15]. According to Cochrane Library, short-term use of Pregabalin decreases pain scores and opiate use but increases adverse events compared to placebo[42].

Pancreatic enzyme replacement therapy: Exogenous enzyme therapy may decrease enzyme secretion and improve malabsorption in patients with exocrine insufficiency. In addition, it is a non-invasive therapy with no adverse effects [43]. However, according to the most recent CP management guidelines, pancreatic enzyme replacement therapy (PERT) is not recommended but is useful for some abdominal symptoms, such as abdominal distension and flatulence in pancreatic exocrine dysfunction[33]. Decreased pancreatic secretion can be used if symptoms persist[44]. In regard to PERT therapy, doses of 1000 USP units of lipase × kg of patient body weight are advised to achieve nutritional parameter improvement[44].

Endoscopic or surgical therapy requires careful patient detection, especially regarding pancreatic anatomy. Patients with pancreatic duct dilatation may benefit from endoscopic or surgical therapies[45]. Therefore, patients may be classified as patients with structural abnormalities (called big-duct disease) and patients without anatomical abnormalities (also called as small-duct disease or minimal change CP)[45].

Endoscopic treatment

The advantages of endotherapy have been largely reported. In fact, endoscopic interventions can be repeated, if required, keeping surgical option valid[46]. International Guidelines[6] recommend ESWL as a safe and effective procedure for uncomplicated painful CP.

Endoscopic complications are divided into early and late complications. Early complications include cholangitis (especially related to sphincterotomy's procedure), pseudocyst infection or pancreatic duct damage[46]. However, endoscopic therapy for CP appears to be a safe and effective option[47]. In the last decade, endoscopic-ultrasound (EUS) guided celiac plexus neurolysis role has been redefined, rediscussing both the technique and patient selection[48].

Surgical treatment

Surgical treatment is recommended for patients when endoscopic treatment has failed for pain relief[33]. Some authors tried to prepare a classification system to establish an international system of pain and QoL surveillance (M-ANNHEIM score)[19].

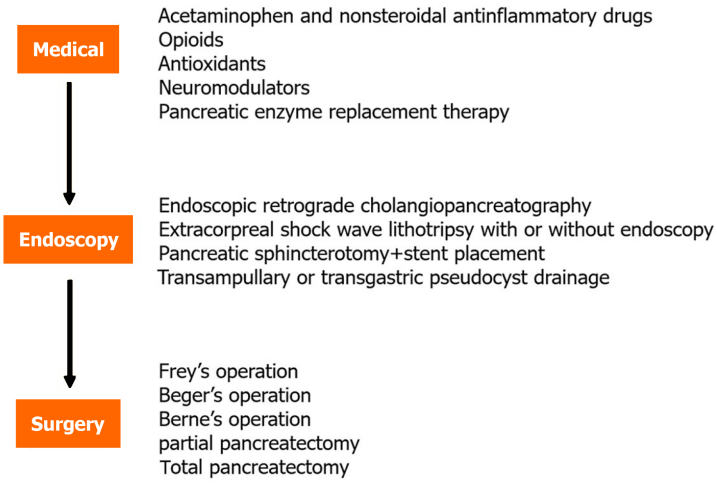

Figure 1 Chronic pancreatitis pain treatment options.

A recent randomized clinical trial (ESCAPE trial) showed that surgical treatment could be more effective than endoscopic first approach for mid-term and long-term pain relief[49]. In this study, a later pancreaticjejunostomy according to Partington and Rochelle is recommended in patients with non-enlarged pancreatic head (< 4 cm). On the other hand, a resection with duodenum preserving is performed for patients with enlarged pancreatic head (> 4 cm)[49].

According to Ratnayake *et al*[50], the Frey procedure is considered the best surgical treatment considering post-operative QoL improvement. It is also considered the procedure with lower complications considering POPF (post-operative pancreatic fistula) and PEI (post-operative exocrine insufficiency).

PAIN TREATMENT COMPLICATED CHRONIC PANCREATITIS

Some important complications may occur in CP

Pancreatic pseudocyst: 20%-40% of CP cases present with pseudocyst. The exact pathogenesis is still unknown. The blockage of the main pancreatic duct and ongoing pancreatic secretion seems to lead to pseudocyst formation[51].

Internal pancreatic fistula: A pancreatic fistula may present both in chronic and acute pancreatitis. It may occur as an asymptomatic cyst or sepsis from infected fluid collection. Minor leaks could be treated in a conservative way. In other cases, an interventional radiologist, skilled endoscopist or a surgeon should be involved[52].

Biliary stenosis: Progressive and irreversible fibrosis of the pancreatic parenchyma in CP leads to benign biliary strictures. In this context, first line therapy is interventional endoscopy with stenting[53].

Pseudoaneurysm: Pseudoaneurysm is a rare complication of CP due to the erosion of peripancreatic vessels by lipolytic and proteolytic enzymes. CP pseudoaneurysms are more common in patients with alcohol abuse[54].

Endoscopic treatment

The aim of an endoscopic approach is to remove obstructing pancreatic obstacles. Endoscopy strategies can achieve therapeutic benefits related to pancreatic outflow obstruction relief to alleviate pain[44]. Significant pain relief can be obtained when ductal irregularities are corrected, stones are extracted and strictures eliminated[44].

All endoscopic interventions are performed by expert endoscopists under consciousness sedation. Some strategies are:

ERCP: Patients with stones and ductal strictures can benefit from drainage procedures[2]. However, ductal stones or strictures often occur in the late stages of disease[45]. They are common both in alcoholic and hereditary pancreatitis. A dilatation with stenting procedure is required or a removal of main duct stones could be chosen in patients with non-enlarged pancreatic head (< 4 cm).

ESWL: It is indicated for disintegrated stones in main pancreatic duct, which are impossible to remove with other endoscopic therapies[19].

According to the 2017 Guidelines[6], ESWL for pancreatic stones is only recommended for ductal stones of 2-5 mm calcified or radiolucent stones. The SCHOKE (Extracorporeal Shock Wave Lithotripsy and Endotherapy for Pain in Chronic Pancreatitis) trial is a randomized controlled trial that demonstrated the effectiveness of external lithotripsy in pancreatic duct decompression and pain relief[55].

Pancreatic sphincterotomy and stent placement for pain relief: An important topic is the role of pancreatic duct stenting in CP. Nowadays, the "on demand stent replacement" instead of "intervals stent replacement" is preferred. The first choice might provide good palliation in CP pain[56].

Transampullary or transgastric drainage of pseudocyst: Pseudocyst drainage should be restricted to patients with important sequelae, such as infection, early satiety and weight loss. According to recent literature, endoscopic pseudocyst treatment has lower mortality and higher success rate than a surgical approach[47]. Both transpapillary and transmural approaches can be used. An EUS-guided transmural approach is preferred for large pseudocysts (d > 5 cm)[47].

All procedures have been studied in adult CP patients and no prospective or randomized controlled trials about CP endoscopic therapy in children have been published[57].

Surgical treatment

Pain represents the most common indication for operative CP management[4]. Some authors consider early surgery as the best choice. According to these authors an early surgical intervention is associated with improved pain control[58]. A proposed cutoff of early surgery is 26.5 mo from symptom onset.

A surgical approach should be suggested: (1) In the 1st 2/3 years after clinical symptoms onset; (2) For patients with five or less endoscopic procedures; and (3) For patients without opioid medical treatment.

Generally, the surgical procedures for pain treatment in CP patients can be divided in: Decompressive procedures, focused on ductal hypertension; Resection procedures, focused on inflammatory masses/stones in the pancreas head. The pancreatic head is the most innervated part of the organ. In this context, surgical removal of pancreatic head results in outflow amelioration. The removal of the inflamed pancreatic head leads to pain relief because it removes the enlarged nerves and improves outflow obstruction[30]. In regard to pancreatic resections, there are many options. The classic Whipple operation or pylorus sparing sacrifices extensive pancreatic resection. Limited pancreatic head resection is involved in Beger's operation and a more extensive drainage procedure is done in Frey operation, combining a longitudinal incision of pancreatic duct and excavation. The Berne procedure (a modified Beger procedure) does not include pancreatic head detachment[59].

In 2022, Waage *et al*[60] generated a CP surgical treatment algorithm considering firstly the presence of pancreatic duct dilatation. DPPHR (duodenum-preserving pancreatic head resection) is necessary in the case of pancreatic duct dilatation with pancreatic head pseudotumor or parenchymal calcification. Among DPPHR, the Frey's procedure is preferred. A pancreatic-jejunostomy is chosen in case of pancreatic duct dilatation but in the absence of pseudotumor/parenchymal calcification[61]. On the other hand, total pancreatectomy procedure is achieved in small duct disease. Distal pancreatectomy with or without splenectomy is indicated for CP tail pathology[60].

According to Skube *et al*[62], Frey's procedure is indicated for patients with main pancreatic duct dilatation and pancreatic head disease. On the other hand, Beger and Berne modification are indicated in patients with pancreatic head or duodenum and/or common bile duct disease involvement.

DISCUSSION

Chronic pancreatitis represents a leading cause of hospitalization. One of the most important and common symptoms related to CP is pain[63]. It usually involves the upper abdomen, often radiating to the back and worsened by meals[16,28].

According to some authors, pain level is also related to CP etiology[28]. In alcohol-induced CP, pain is a constant symptom, while in "senile" or delayed-onset CP, the painless course is more frequent (50%). According to Amman *et al*[64], two different patterns are recognised: Type A: Characterised by recurrent episodes of abdominal pain; Type B: Characterised by prolonged or persistent pain.

Type A is characterised by short periods of pain and long pain-free intervals. Patients with type A pain are managed medically. On the other hand, type B has been hypothesised to be due to local complications, needing surgical intervention to achieve pain relief[65]. Completely painless chronic pancreatitis is a very rare form of CP[8].

However, according to a recent study by Kempeneers *et al*[66], the continuous and intermittent pain patterns in CP seem not to be two different pathophysiological entities. In fact, no differences in imaging and disease duration have been highlighted. According to the same study, different sub-patterns can be identified in the continuous: Persistent pain with slight fluctuation, persistent pain with pain attacks and pain attacks with pain between them. All of these different pain patterns can be mixed with each other.

At the beginning of 2000, CP mechanisms have been largely analysed and two different theories have been proposed: The neurogenic theory and the intraductal/intraparenchymal hypertension theory. According to the first one, CP is generated by a result of increased pressures, like in compartment syndrome. While, according to the second one, pain is generated by noxious substances on peripancreatic nerves[67]. However, nowadays, the complexity of CP is well recognised.

The most important effect of pain is the worsening of QoL. Psychiatric comorbidities are prevalent in CP patients. The effect of anxiety seems to be mediated *via* pain, while depression is independently related to QoL[68,69].

Pain has a central role in CP treatment[70]. An increasing number of studies elucidated the efficacy of a mechanism-based-treatment with specific analgesic protocol[71].

The optimal management of CP involves several specialties and, similar to cancer patients, may benefit from a multidisciplinary team[60,72].

In the past, many different surgical approaches to CP pain treatments have been proposed, such as the DuVal procedure, involving pancreatic tail resection with splenectomy followed by pancreatic jejunostomy, in order to improve retrograde drainage and pain[4]. However, a conservative step-up approach is currently considered the gold standard [73].

Alcohol and smoking cessation is suggested and a low-fat diets is also useful[37]. The medical treatment is considered the first approach[74]. The "three-step ladder" is largely adopted in this context. The first medical step is NSAID use. Opioid analgesics are commonly used, but they cannot be used for long-term treatment protocols, because of dependence risk and complications[37]. A combined antioxidant therapy seems to be safe and effective in CP pain relief[75].

A non-conservative approach is then attempted in case of conservative treatment failure. Endoscopy is considered a good alternative to surgery since the early years of 2000[76]. During the last 2 decades, the advancement of pancreatic endotherapy has given a significant contribution to the management of pancreatic pain.

In CP, main pancreatic duct obstruction caused by stricture or stones or by a combination of both requires interventional endoscopy or surgical approach[56]. These interventions and decreasing intraductal pancreatic pressure, can provide pain relief[77]. Pain represents the most common indication for operative CP management[4].

Among endoscopic therapies, an ERCP including dilatation with stenting procedure is required. An expertise endoscopist is recommended[2]. In case of big stones with diameter greater than 2-5 mm, the ESWL approach can be chosen. ERCP and ESWL both have great results in pain relief[19].

On the other hand, the surgical approach involves decompressive or resection procedures. The first ones focus on ductal hypertension, while the second ones focus on inflammatory masses/stones. Among surgical procedures, the Frey operation combines a longitudinal incision of pancreatic duct and parenchyma excavation, the Beger procedure is a limited pancreatic head resection, while Berne modification procedure involves a more limited pancreatic head resection [62]. In case of intraductal papillary mucinous neoplasm or suspected malignancy, a partial pancreatectomy is indicated. In the case of intractable disease, hereditary pancreatitis or small duct disease a total pancreatectomy should be necessary [62].

Some observational studies have suggested that the early surgery could reduce a disease progression, preserving pancreatic function[49].

An important challenge is the difficulty to compare different treatment efficacies in pain relief because of the lack of an international scale for pain comparison[11]. However, the Pancreatitis-Quantitative Sensory Testing consortium is working on meta-analysis comparing endoscopic and surgical treatments[17].

A recent systematic review including only randomized clinical trials comparing short-term and long-term outcomes showed superior results in surgical interventions compared to endoscopic ones. The number of complications is similar in both groups[77]. However, no definitive or international consensus has been achieved.

The new frontiers of interests in CP pain treatment have been reported in an article by Maydeo *et al*[78]. Being less invasive with acceptable complications, they prefer endoscopic approaches as first treatment. They also consider endotherapy the best in cost-effectiveness, because of biodegradable stents that reduce overall cost.

CONCLUSION

In conclusion, CP pain management is an ongoing challenge. Many different mechanisms are involved in CP pain onset. A tailored treatment for each patient allows for faster and effective pain control. Much progress has been made in CP pain comprehension and treatment, but the lack of international treatment protocols remains a major problem[79]. Nowadays, a step-up tailored treatment discussed in a multidisciplinary setting is considered the gold standard.

FOOTNOTES

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Country/Territory of origin: Italy

ORCID number: Margherita Binetti 0000-0003-2630-5884; Valeria Tonini 0000-0003-3130-2928.

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Method "Monte Carlo" in healthcare

Tsvetelina Velikova, Niya Mileva, Emilia Naseva

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Tsvetelina Velikova, Medical Faculty, Sofia University St. Kliment Ohridski, Sofia 1407, Bulgaria

Niya Mileva, Medical Faculty, Medical University of Sofia, Sofia 1431, Bulgaria

Emilia Naseva, Faculty of Public Health "Prof. Tsekomir Vodenicharov, MD, Dsc," Medical University of Sofia, Sofia 1431, Bulgaria

Corresponding author: Emilia Naseva, PhD, Senior Scientist, Faculty of Public Health "Prof. Tsekomir Vodenicharov, MD, DSc," Medical University of Sofia, 15 Acad I.E. Geshov Blvd, Sofia 1431, Bulgaria. e.naseva@foz.mu-sofia.bg

Abstract

In public health, simulation modeling stands as an invaluable asset, enabling the evaluation of new systems without their physical implementation, experimentation with existing systems without operational adjustments, and testing system limits without real-world repercussions. In simulation modeling, the Monte Carlo method emerges as a powerful yet underutilized tool. Although the Monte Carlo method has not yet gained widespread prominence in healthcare, its technological capabilities hold promise for substantial cost reduction and risk mitigation. In this review article, we aimed to explore the transformative potential of the Monte Carlo method in healthcare contexts. We underscore the significance of experiential insights derived from simulated experimentation, especially in resource-constrained scenarios where time, financial constraints, and limited resources necessitate innovative and efficient approaches. As public health faces increasing challenges, incorporating the Monte Carlo method presents an opportunity for enhanced system construction, analysis, and evaluation.

Key Words: Monte Carlo; Simulation; Healthcare; Modeling; Decision analysis; Stochastic methods; Statistical techniques; Health economics

Core Tip: The potential of the Monte Carlo method in healthcare spreads across decision-making, risk analysis, and modeling in healthcare. Emphasizing versatility, the method navigates uncertainties, offering insights for optimal resource allocation, cost-effectiveness evaluations, and strategic planning in the healthcare domain. The Monte Carlo technique could be demystified through clear illustrations and real-world examples, empowering practitioners to harness its power for robust analyses, enhancing decision accuracy, and contributing to improved healthcare strategies and outcomes.

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INTRODUCTION

In recent years, the Monte Carlo method has emerged as a powerful and versatile tool in healthcare research, revolutionizing how we approach complex problems[1]. Originating from statistical physics, this computational technique is increasingly applied to model intricate healthcare scenarios, offering a sophisticated approach to decision-making and analysis[1].

Analytical modeling involves preparing and using simulation tools to solve real-world problems. Simulation is based on a large group of methods and applications for imitating the behavior of real systems, usually through a computer and appropriate software[2]. Computer simulation allows us to make a computer representation of a real system and to experiment with the computer version. In this way, the behavior of the real system in different situations can be better understood and predicted[2].

However, simulation is particularly valuable for solving problems that cannot be solved by analytical mathematical approaches and for problems that involve random variables[3].

There has been a growing interest in using simulation (imitation) models in various fields in the last few decades. In business, they are primarily used to support analysis and decision-making under conditions of uncertainty and risk[4]. One of the approaches to account for the uncertainty of the business environment, respectively the risk, when preparing calculations in the field of investment analysis, financial analysis, and a number of other areas of business analysis is the use of stochastic (probability) models. Stochastic models' input variables (key factors) are random variables whose behavior is beyond decision-makers control[4-6]. These models can also be successfully used in the field of healthcare.

The Monte Carlo method simulates various possible outcomes using random sampling and statistical analysis[7]. In healthcare, this methodology has proven invaluable in treatment planning, risk assessment, and resource allocation. For instance, in cancer treatment, Monte Carlo simulations enable the exploration of various radiation therapy scenarios, optimizing dose delivery and minimizing adverse effects[7].

Furthermore, Monte Carlo simulations find applications in health economics, helping researchers evaluate cost-effectiveness and assess the economic impact of different healthcare interventions[5]. The method's ability to account for uncertainties and variability makes it particularly useful when dealing with intricate, dynamic systems inherent in healthcare[8].

As we navigate the complexities of modern healthcare, the Monte Carlo method offers a unique and powerful approach to enhance decision-making processes and refine our understanding of intricate medical phenomena. This review aims to provide a comprehensive overview of the application and practical utility of the Monte Carlo method in healthcare, emphasizing its role in the applications, benefits, and potential pitfalls of employing it in healthcare research, including decision-making, risk analysis, and modeling. Moreover, the Monte Carlo approach could shed light on its transformative role in shaping the future of medical investigation and decision support systems.

SEARCHSTRATEGY

To conduct a thorough review, we employed a systematic search strategy across the main databases, including PubMed, Scopus, and Medline, from inception to 14 February 2024. The search utilized a combination of relevant keywords, such as "Monte Carlo method," "healthcare," "simulation," "decision analysis," "modeling," "stochastic methods," "statistical techniques," and "health economics." Boolean operators (AND, OR) were strategically applied to refine the search and capture the breadth of literature on applying the Monte Carlo method in healthcare settings. Additionally, reference lists of critical articles were manually scanned to ensure inclusiveness.

BASICSOF MONTE CARLO METHODOLOGY

Principles and concepts

The purpose of simulation is to simplify reality so that we can better understand it. Simulation is better than experiments because it "compresses" time and removes unnecessary details. Unlike actual processes, simulation is used for

optimization and training[2,9]. The other feature of the simulation is that it is dynamic and active. Simulation involves creating a model of a system, conducting experiments with it, and analyzing the results to be applied to the actual system later. The purpose of these what-if experiments is to determine how the real system works and to predict the effect of changes on the system over time[8,9].

For example, business simulation is used to provide answers to the following questions: (1) Will the change in process increase yield/productivity/quality/revenue? (2) How many people are needed to maintain services at a certain level? (3) Can we create a system with a few components and keep it stable simultaneously[8,9]?

Development (construction) of a model of a system, usually mathematical and logical in nature, as well as actual or theoretical (virtual) includes the following stages: describing the real system in terms acceptable to computer systems; using a computer to run a simulation; and mimicking the action of the real system/process[3]. Since the simulation could also be considered an experiment, the goal is to find elements related to the real system, *i.e.* modeling and mimicking an actual process that can be modified by the simulation performer[3].

Mathematical procedures for modeling complex problems that cannot be solved theoretically are known as the Monte Carlo method[10]. The name of these techniques comes from the research of nuclear reactions at the beginning of the Second World War, when a solution to the problem of whether it was possible to induce a nuclear reaction was sought [10]. It was known that multiple neutrons moving in uranium could randomly cause the subsequent emission of other neutrons. Still, it was impossible to predict theoretically whether the chains of reactions forming a complex network would cause an atomic explosion or the prepared high explosive would break on the surface. The scientists investigating this problem used the first large computer ever built to model the random trajectories of neutrons through the atoms of the uranium charge. The project was classified under the code name "Monte Carlo." Its name was chosen because of the similarity of statistical simulation to games of chance in Monaco's capital, the European gambling center[10].

Because this significant project was the first to use a computer and the theory of random trajectories to obtain a probabilistic solution to a complex physical problem, these mathematical experiments were called "Monte Carlo" methods[11]. McCracken, in 1955, when presenting the early "Monte Carlo" methods in the Journal of the American Statistical Association, wrote that "Monte Carlo" method is mainly used to solve problems that are determined in some critical way probabilistically-tasks where physical experiment is infeasible and the creation of an exact formula is impossible[11]. American mathematicians Metropolis and Ulam[10] are recognized as the inventors of the method in 1949.

From then on, Monte Carlo simulation became the primary technique for studying and modeling high uncertainty and risk events. The method is widely used in various scientific research and practice fields-from space exploration to predicting business bankruptcies and risk.

Furthermore, the "Monte Carlo" method is a universal simulation method with several healthcare applications[12]. The main advantages of the approach are its accuracy (builds a complete picture of risk), flexibility (allows risk managers to use different theoretical distributions and dynamic correlations), universality and possibility of integration in different risk modules[12].

The main drawbacks of "Monte Carlo" stem from the heavy computational procedure, requiring a considerable number of simulations (minimum 10000 simulations fora risky asset) and insufficient time to re-evaluate large bank portfolios under dynamic changes in financial markets[5]. The main idea of Monte Carlo simulation in economics is to construct a detailed picture of portfolio risk by computer simulation of many random numbers possessing the main characteristics of the empirical distribution of portfolio returns over a certain period[5].

Computer simulation models, including the Monte Carlo method, have advantages and disadvantages (Table 1).

APPLICATIONSOF THE MONTE CARLO METHOD IN HEALTHCARE

From a theoretical point of view, the Monte Carlo method can be thought of as a technique of numerical integration of a single random variable to deal with non-determinism[13]. An essential feature of the method is that the standard error decreases only with the square root of the model size, not the model's size.

Monte Carlo simulation is essentially a numerical method, primarily described as a statistical simulation method. A statistical simulation could be any method that uses a sequence of random numbers to represent the simulation[14]. A simulation can typically involve over 10000 model evaluations using supercomputers. The Monte Carlo method is one of many methods for analyzing the distribution of uncertainty, where the goal is to determine how random variation, lack of knowledge, or error affects the sensitivity, performance, or reliability of the modeled system[13]. Monte Carlo simulation is categorized as a sampling method because the inputs are arbitrarily generated from the probability distribution to simulate the sampling process from the actual population. The data generated by the simulation can be presented as probability distributions (or histograms) or converted into error bounds, reliability estimates, tolerance zones, and confidence intervals[14].

One of the earliest applications of the Monte Carlo method in medicine was in risk analysis for human tetrachlorethylene carcinogenicity using no pharmacokinetic models[15]. The authors treat the parameters of the pharmacokinetic model as random variables and determine the bounds of the risk estimates after accounting for parameter uncertainty through Monte Carlo simulations. They further assessed the sensitivity of the pharmacokinetic model predictions to its parameters by analyzing the results of Monte Carlo simulations, demonstrating that the kinetic parameters that define the percent metabolized tetrachlorethylene are the most important for assessing the risk of carcinogenicity in humans[15].

Population pharmacokinetic modeling coupled with Monte Carlo simulations has proven a powerful tool for science-based decision-making. Early stages in the clinical evaluation of new drugs face three critical issues: (1) Extrapolation of

Table 1 What computer simulation models allow and what cannot

Advantages[8,9]	Limitations[2,3]
Allow conclusions to be drawn about a new system without having actually to build it or to make changes to an existing one without disrupting its operation	Cannot optimize but can only generate results from "WHAT-IF" queries
Allow the manager to visualize the operations of a new or existing system under different conditions	Cannot obtain correct results from inaccurate data
Allow us to see how different components interact and how this affects the overall system performance	Cannot describe system characteristics that were not included in the model
Allow general insight into the essence of the process	Cannot solve problems; they can only provide information that aids the process of developing a solution
Allow recognition of specific problems and problem areas in the studied system	Cannot give simple answers to complex problems
Assist in the development of particular policies and process plans	
Improve system efficiency	

preclinical data to humans; (2) Safety and tolerability concerns regarding dosage; and (3) Scientifically based drug combinations[13]. The Monte Carlo method has shown great promise in drug development, particularly in designing phase II/III clinical trials of antimicrobial agents and the appropriate dosage prescribed for humans[16]. In real life, interindividual variability in the pharmacokinetic values of a given drug cannot be excluded or ignored. Furthermore, there is a spectrum of variable susceptibility to each test drug among microorganisms of clinical interest. Therefore, any method for examining the adequacy of a fixed-dose regimen must also explicitly account for sources of variability (pharmacokinetic and microbiological). The method used by the authors consists of applying data from preclinical microbiological and animal models together with data from early phase I pharmacokinetic studies[13].

The Monte Carlo method is also flexible enough to assess the impact of changing dosage. It can also accurately compare drugs from different classes (*e.g.*, fluoroquinolones with macrolides or beta-lactams with aminoglycosides) because different targets are set for each drug class[13].

The third major issue in drug development is the reliable judgment of doses and the scheme of combining with other drugs. The ability to assess the impact of both dose and regimen in combination with other medications can streamline the search for an optimal dose regimen to be studied in clinical experience in the shortest possible time and with the smallest possible number of patients[17]. Integrating population pharmacokinetic modeling with the Monte Carlo simulation method offers a new opportunity to develop optimal and more reliable alternative dosing strategies with antibacterial drugs (*i.e.* beta-lactams) to achieve a predefined therapeutic goal. Alternative regimens optimize dosing with a lower total daily dose than would be used with traditional dosing methods[17,18].

A promising area of application of the Monte Carlo method is the emerging approach of simulating clinical trials to maximize the information gained during the drug development process to achieve the most significant success in clinical trials[19]. From a financial perspective, simulation allows pharmaceutical companies to reduce the number of studies required, maximize the chances of success in clinical trials and possibly shorten development time. All these results will reduce the cost of drug development[19].

The significant clinical benefit of the nonparametric population pharmacokinetic modeling approach is that multiple reference points, with their various sets of pharmacokinetic parameter values, provide multiple predictions of future plasma concentrations and other responses from future doses[13]. The ultimate benefit of Monte Carlo simulations are seen in drug phenotyping when dealing with clinical data that are inherently limited and sparsely distributed[13].

The method of Monte Carlo also serves as a useful tool in the pursuit of precision medicine, empowering clinicians and researchers to navigate the complexities of individualized patient care and enhance drug efficacy in diverse clinical scenarios[20]. By simulating the diverse biological and physiological factors influencing drug response, Monte Carlo simulations enable researchers to tailor treatment strategies to the unique needs of patients, optimizing therapeutic effectiveness while minimizing adverse effects[20]. Furthermore, Monte Carlo techniques facilitate the exploration of alternative dosing regimens, drug combinations, and patient stratifications, fostering a more precise and personalized approach to healthcare delivery. The Monte Carlo method simulates the dynamic interactions between pharmaceutical compounds, biological systems, and patient-specific factors to predict and optimize treatment outcomes in drug efficacy modeling[20]. By incorporating parameters such as drug pharmacokinetics, pharmacodynamics, and individual patient characteristics, the method simulations provide a comprehensive framework for assessing drug efficacy in diverse populations. For instance, researchers can utilize Monte Carlo techniques to model the distribution of drug concentrations in different tissues and organs over time, accounting for variability in absorption, distribution, metabolism, and excretion processes[20]. This enables the exploration of optimal dosing regimens to achieve therapeutic concentrations while minimizing toxicity risks. Moreover, by simulating the effects of genetic polymorphisms, comorbidities, and concomitant medications, researchers can assess interindividual variability in drug response and identify factors influencing treatment efficacy and safety[21]. Through Monte Carlo simulations, researchers can also conduct virtual clinical trials to assess the impact of different treatment protocols, dosage adjustments, and patient stratifications on therapeutic outcomes. The method has been applied to models and simulates drug development for various pathologies, such as thromboembolism, diabetes, rheumatoid arthritis, *etc*[21]. Monte Carlo methods also facilitate risk assessment and stratification by estimating the probability of specific clinical events, such as disease recurrence, complications, or mortality, based on individual

patient characteristics and treatment interventions. By simulating large populations of virtual patients with varying risk profiles, Monte Carlo simulations enable the identification of high-risk subgroups and the development of targeted interventions to mitigate adverse outcomes. This enables clinicians to anticipate disease milestones, evaluate treatment effectiveness, and inform patient management strategies[21].

The method may serve as a cornerstone in economic impact assessments of interventions and treatments, offering a robust computational framework to evaluate the potential economic consequences of healthcare policies and medical innovations[5]. Through iterative sampling of key variables such as treatment costs, healthcare utilization, and productivity gains, Monte Carlo techniques provide probabilistic estimates of economic outcomes, including cost-effectiveness, budget impact, and return on investment. These simulations facilitate sensitivity analyses to assess the robustness of economic evaluations to variations in input parameters and model assumptions, enhancing the credibility and transparency of decision-making processes[5]. In essence, Monte Carlo simulations are pivotal in advancing evidence-based healthcare decision-making by quantifying the economic implications of interventions and treatments, ultimately guiding policymakers, payers, and healthcare stakeholders toward more informed and efficient resource allocation strategies.

Some of the major fields where the method Monte Carlo takes part are presented in Table 2[22-26].

CHALLENGESAND LIMITATIONS OF THE MONTE CARLO METHOD

Sensitivity to input parameters

The results of the Monte Carlo simulation are sensitive to input parameters. Thus, data quality is crucial. The input data have to be reliable. In addition, they should cover as long a period of time as possible[27].

Another challenge regarding the data is their distribution. Most of the phenomena generally follow Gaussian distribution, but not all of them. Datasets covering longer periods could help reveal the distribution's true shape. If the distribution cannot be identified based on available data, then a goodness-of-fit technique could be used. Further bootstrapping could be performed if the distribution is still unknown. The last option proposed by the literature is to use the "default" distributions: normal, log-normal, and uniform[27].

Computational demands

Increasing the number of input parameters increases the accuracy of the simulation. From a certain point forward, adding additional variables makes the analysis harder and time-consuming, even with the help of computer programs. There are different techniques for variable selection[28].

Monte Carlo simulation requires using specialized software such as MATLAB or add-ins for Excel. More than 20 years ago, Brian O'Connor wrote syntax codes for creating simulations for the most popular statistical software packages[29]. Thus, the Monte Carlo simulation could be performed not only by mathematicians.

Ethical considerations

Due to ethical reasons, not all types of experimental studies in the field of medicine and healthcare can be performed. The simulations fill in that gap in the knowledge and provide valuable information for unknown parameters that could not be studied in reality.

The conclusions from the Monte Carlo method application are based not on actual data but on simulations. There might be limitations in the design of the simulation, the selection of variables and their distribution. This means that the results applied in the practice could also affect human health in a negative aspect.

FUTUREDIRECTIONS AND INNOVATIONS

As the healthcare landscape continues to evolve, future directions for applying the Monte Carlo method in healthcare simulation hold great promise. Advancements in computational power are anticipated to play a pivotal role, enabling the development of more intricate and realistic models that capture the complexity of healthcare systems. The integration of artificial intelligence (AI) represents a paradigm shift, where machine learning algorithms enhance Monte Carlo simulations' adaptability and predictive capabilities. This synergy empowers healthcare professionals to derive actionable insights from vast datasets, facilitating personalized treatment strategies and resource optimization. Furthermore, the Monte Carlo method is poised to extend its reach into emerging healthcare fields, such as precision medicine, genomics, and digital health[30]. Innovations in simulation techniques will likely contribute to refining decision-making processes, policy formulation, and healthcare planning. The convergence of computational power, AI, and the expanding horizons of healthcare applications positions the Monte Carlo method as a dynamic and indispensable tool in shaping the future of evidence-based healthcare practices.

CONCLUSION

The benefit of simulation modeling is in evaluating new systems without having to build them, experimenting with

Table 2 Application of Monte Carlo method in cancer treatment optimization, personalized medicine and drug efficacy modeling, predictive modeling for disease outcomes, evaluation of treatment risks and benefits, and economic impact assessment of interventions and treatments

Field	Specific issue	Outcomes	Ref.
Cancer treatment optimization	Radiation therapy simulations	Applications of the Monte Carlo method to model treatment heads for neutral and charged particle radiation therapy and specific in-room devices for imaging and therapy purposes	Park *et al* [22], 2021
	Dose delivery strategies	The method may be used to calculate dose distributions and further investigations aimed at improving dose delivery and planning in cancer patients	Chiuyo *et al* [23], 2022
Personalized medicine and drug efficacy modeling	Antibiotic dosing regimen analysis	The simulated therapeutic curve was virtually identical to that obtained experimentally	Milligan *et al*[21], 2013
Predictive modeling for disease outcomes	Infectious disease	The employed Bayesian Monte Carlo regression framework allows for incorporating prior domain knowledge, which makes it suitable for use on limited yet complex datasets as often encountered in epidemiology	Stojanović *et al*[24], 2019
	COVID-19	The method of the Monte Carlo algorithm was used to conduct Bayesian inference and illustrate the proposed approach with data on COVID-19 from 20 European countries. The approach performs well on simulated data and produces posterior predictions that fit reported cases, deaths, and hospital and intensive care occupancy well	Rehms *et al* [25], 2024
Evaluation of treatment risks and benefits	Application to the prophylaxis of deep vein thrombosis	The simulation was feasible to model the joint density of therapeutic risks and benefits of prophylaxis in patients with deep vein thrombosis	Lynd *et al* [26], 2004
Economic impact assessment of interventions and treatments	A Monte Carlo simulation approach for estimating the health and economic impact of interventions provided at SRCs	Using Monte Carlo simulation methods, the health and economic impact of SRCs can be reasonably estimated to demonstrate the utility of SRCs and justify their growing importance in the healthcare delivery landscape of the United States	Arenas *et al* [5], 2017

COVID-19: Coronavirus disease 2019; SRCs: Student-run clinics.

existing systems without changing them, and testing the limits of systems without destroying them, *i.e.* simulation can be used to construct, analyze and evaluate various systems.

We can formulate the following take-home messages: (1) Through analytical modeling, one could understand and predict the behavior of a real system in various situations and problems, especially those that cannot be solved by mathematical approaches or involve quantities of a random nature; (2) Simulation is better than experiment because its "compresses" time and removes unnecessary details. Moreover, it is dynamic and active; (3) Simulation involves creating a model of a system, conducting experiments with it, and analyzing the results to later apply to the real system; (4) Mathematical procedures for modeling complex problems that cannot be solved theoretically are known as the "Monte Carlo" method; (5) The approach's main advantages are its accuracy (building a complete risk picture), flexibility (allowing risk managers to use different theoretical distributions and dynamic correlation dependencies), universality and the possibility of integration into different risk modules; (6) The main drawbacks of "Monte Carlo" stem from the heavy computational procedure, requiring a considerable number of simulations (minimum 10000 simulations for a risky asset) and insufficient time for re-estimation under dynamic changes in financial markets; and (7) One of the earliest applications of the Monte Carlo method in medicine was in risk analysis of carcinogenicity of certain drugs in humans, in the design of clinical trials phase 2/3 in clinical studies, for accurate comparison of drugs of different classes, combining with other medications, and other.

The Monte Carlo method is not yet a mainstream tool in healthcare, but it is a technology with immense potential to reduce cost and minimize risk. Experience gained through simulated experimentation is critical in situations of limited time, money, and resources.

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FOOTNOTES

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Country of origin: Bulgaria

ORCID number: Emilia Naseva 0000-0002-1282-8441.

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ORIGINAL ARTICLE

Case Control Study

Role of lymphocyte-to-monocyte ratio as a predictive marker for diabetic coronary artery disease: A cross-sectional study

Pradeep Kumar Dabla, Dharmsheel Shrivastav, Pratishtha Mehra, Vimal Mehta

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Pradeep Kumar Dabla, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Delhi 110002, India

Dharmsheel Shrivastav, Department of Biochemistry, Govind Ballabh Pant Institute of Post Graduate and Medical Research, Delhi 110002, India

Pratishtha Mehra, Vimal Mehta, Department of Cardiology, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Delhi 110002, India

Corresponding author: Pradeep Kumar Dabla, MD, Professor, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research Delhi, J.L.N Marg, Delhi 110002, India. pradeep_dabla@yahoo.com

Abstract

BACKGROUND

The lymphocyte to monocyte ratio (LMR) is considered a marker of systemic inflammation in cardiovascular disease and acts as predictor of mortality in coronary artery disease.

AIM

To investigate the predictive role of LMR in diabetic coronary artery disease patients.

METHODS

This cross-sectional study was conducted at tertiary care super-specialty hospital at New Delhi, India. A total of 200 angiography-proven coronary artery disease (CAD) patients were enrolled and grouped into two categories: Group I [CAD patients with type 2 diabetes mellitus (T2DM) and glycated hemoglobin (HbA1c) levels ≥ 6.5%], and Group II (CAD patients without T2DM and HbA1c levels < 6.5%). Serum lipoproteins, HbA1c, and complete blood count of enrolled patients were analyzed using fully automatic analyzers.

RESULTS

The logistic regression analysis showed an odds ratio of 1.48 (95%CI: 1.28-1.72, $P < 0.05$) for diabetic coronary artery disease patients (Group I) in unadjusted model. After adjusting for age, gender, diet, smoking, and hypertension history, the odds ratio increased to 1.49 (95%CI: 1.29-1.74, $P < 0.01$) in close association with LMR.

Further adjustment for high cholesterol and triglycerides yielded the same odds ratio of 1.49 (95%CI: 1.27-1.75, $P < 0.01$). Receiver operating characteristic curve analysis revealed 74% sensitivity, 64% specificity, and 0.74 area under the curve (95%CI: 0.67-0.80, $P < 0.001$), suggesting moderate predictive accuracy for diabetic CAD patients.

CONCLUSION

LMR showed positive association with diabetic coronary artery disease, with moderate predictive accuracy. These findings have implications for improving CAD management in diabetics, necessitating further research and targeted interventions.

Key Words: Coronary artery disease; Type 2 diabetes mellitus; HbA1c; Lymphocyte to monocyte ratio; Lymphocyte to monocyte ratio

Core Tip: The lymphocyte to monocyte ratio (LMR) proves to be a potential marker of systemic inflammation in cardiovascular disease, demonstrating a predictive role in mortality among diabetic coronary artery disease (CAD) patients. This cross-sectional study investigates the predictive capacity of LMR specifically in individuals with diabetic coronary artery disease. The results reveal LMR as a contributing factor in diabetic CAD, with its moderate predictive accuracy. The study underscores the potential clinical relevance of LMR in improving CAD management in diabetic patients, urging further research and targeted interventions for enhanced patient outcomes.

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INTRODUCTION

In the general population, the coronary artery disease (CAD) is a major cause of morbidity and mortality worldwide. In India, the prevalence of cardiovascular disease among adults ≥ 45 years was 5.2% in 2019[1]. The hyperglycemia contributes significantly to the development of cardiovascular disorders[2]. Chronic inflammation plays a key role in each phase of coronary artery disease, from endothelial dysfunction and plaque disruption to manifestation of clinical signs and symptoms associated with acute atherothrombotic events[3]. Several studies have suggested that chronic inflammation may be associated with increased risk of atheromatous disease, including CAD and stroke and type 2 diabetes mellitus (T2DM)[4]. Among the different markers of inflammation, including leukocyte count and different subtypes of white blood cell (WBC) counts such as neutrophils, monocytes, and lymphocytes, there is a correlation with an elevated risk of cardiovascular events[5]. The published literature shows that the ratios of subtypes of WBCs including neutrophil to lymphocyte and lymphocyte to monocyte ratio have a significant association with the incidence and severity of CAD [6]. Thus, it can be an important factor to assess the prognosis of the patient.

The lymphocyte-to-monocyte ratio (LMR) is a newly recognized systemic inflammatory marker and demonstrated its usefulness as an indicator of the systemic inflammatory response and its potential as a prognostic factor in different types of cancer and cardiac diseases[7]. The emerging evidence from the experimental and clinical studies supports that LMR could be an independent risk factor for CAD[8]. The LMR has also been shown to correlate with the in-hospital death rate among patients experiencing acute type A aortic dissection[9]. Similarly, some studies have indicated a relationship between LMR and cardiovascular disease as well as adverse cardiovascular events[10-12]. However, there is paucity of data regarding the relationship of LMR in diabetic and non-diabetic CAD. Thus, the aim of this study is to investigate the association of LMR with diabetic CAD patients compared with non-diabetic CAD patients.

MATERIALS AND METHODS

Study design and population

This cross-sectional study was conducted at the Department of Biochemistry at G.B. Pant Institute of Postgraduate Medical Education and Research in New Delhi, India. A total of 200 patients with angiographically proven CAD were enrolled from Cardiology outpatient and in-patient department. The study was conducted in accordance with internationally accepted recommendations for clinical investigation (Declaration of Helsinki of the World Medical Association, revised October 2013). The study was approved by the institutional ethical committee of Maulana Azad Medical College and associated hospitals, Delhi, India.

Inclusion and exclusion criteria

The inclusion criteria for the study were adult patients aged over 18 years, regardless of gender, who had been diagnosed with coronary heart disease based on resting electrocardiography and invasive coronary angiography with more than 50% stenosis in at least one coronary artery[13]. On the other hand, patients below the age of 18 years, patients with renal and hepatic impairment, patients who had undergone previous procedures such as coronary artery bypass grafting, percutaneous transluminal coronary angioplasty, and stenting were excluded from this study.

Demographic characteristics

The study subjects were required to complete a questionnaire to gather demographic information related to their age, gender, dietary habits, addictive habits, and history of diabetes, hypertension. Additionally, for patients with T2DM, the questionnaire also collected information regarding, duration of diabetes and symptoms experienced at the time of diagnosis.

Sample collection and processing

Venous blood samples were collected from each participant under proper aseptic conditions. Three milliliters of blood were transferred to an EDTA vial for glycated hemoglobin (HbA1c) analysis, and the remaining sample was transferred to a citrate vial for blood sugar analysis. The enrolled patients were categorized into two groups based on their previous diabetes diagnosis and HbA1c levels: Group I consisted of patients with HbA1c levels ≥ 6.5% and a history of diabetes, while Group II consisted of individuals without a history of diabetes and HbA1c levels below 6.5%[6]. Lipid profile and complete blood count were measured by fully automatic analyzers.

The atherogenic indexes were calculated using the following formulas: NHC [non-high density lipoprotein (HDL) cholesterol] = Serum total cholesterol-serum high-density lipoprotein cholesterol (HDL-C). AC (Atherogenic coefficient) = Non-high-density lipoprotein cholesterol (NHC)/serum HDL-C. AIP (Atherogenic index of plasma) = Log (serum triglyceride/serum HDL-C). CRI-I (Castelli's Risk Index I) = Serum total cholesterol/serum HDL-C.CRI-II (Castelli's Risk Index II) = Serum low density lipoprotein cholesterol/serum HDL-C.

Statistical analysis

The data was analyzed using the statistical program for social science (SPSS) version 21, IBM Corp., Chicago, United States. The normality of the data was checked using the Shapiro-Wilk test. Student *t*-tests, ANOVA, and Mann-Whitney *U* tests were used to compare parametric and non-parametric variables, while binary logistic regression tests were applied to determine the odds ratio analysis. Receiver operating characteristic (ROC) curve analysis is conducted to evaluate the significance and effectiveness of the LMR in assessing the role of LMR in diabetic patients with coronary artery disease. All statistical tests were considered significant at a level of $P < 0.05$.

RESULTS

The mean age of Group I (81 males and 19 females) and Group II (89 males and 11 females) were 54.2 ± 10.2 and 53.2 ± 10.3 years, respectively. Regarding dietary habits, most of the patients were non-vegetarian, 81 (81%) patients in Group I and 60 (60%) in Group II, $P = 0.004$. For addiction, the frequency of smokers were significantly higher in Group I (60%) compared with Group II (25%). Regarding history of hypertension, Group I had significantly higher ($P = 0.003$) number of hypertensive patients (39%) than Group II (20%). In biochemical parameters, the diabetes specific parameters *i.e.*, HbA1c and random blood sugar levels were significantly higher ($P < 0.001$) in Group I. Further we observed significant differences ($P < 0.05$) in serum levels of total cholesterol (TC), triglycerides (TG), and very low-density lipoprotein cholesterol (VLDL) and non-HDL cholesterol (non-HDL-C) between Group I and Group II. In terms of white blood cell counts, Group I had significantly higher lymphocyte counts (3.49 ± 1.68 × 10^9/L) and LMR (6.19 ± 4.14) than Group II (2.46 ± 1.27 × 10^9/L) and LMR (4.19 ± 2.78), ($P = 0.001$, for both).

The comparison of demographic characteristics and biochemical parameters is given in Table 1. On comparison of lipid indices, the AIP, AC and CRI-I were significantly higher ($P < 0.001$) in Group I compared with Group II (Table 2). Logistic regression was used to estimate the odds ratio for CAD in T2DM; in unadjusted conditions the LMR increased the risk of CAD in T2DM, odd ratio 1.48 (95%CI: 1.28-1.72, $P = 0.01$). Further on adjusted cofounding variables model-1 (age, gender, diet, smoking, history of hypertension), the LMR increases the risk of CAD in T2DM with the odds ratio 1.49 (95%CI: 1.29-1.74, $P = 0.001$). Also, on adjusting the variable model 1 along with high TC and high TG, the odds ratio was 1.49 (95%CI: 1.27-1.75, $P = 0.001$) (Table 3). Furthermore, we observed the ROC curve analysis for estimating the threshold cut-off value of LMR for CAD with T2DM was 4.3. The area of the curve is 0.74 (95%CI: 0.67-0.80, $P < 0.001$) with sensitivity of 72% and specificity of 64%. The cut off value was 4.3 for LMR in diabetic coronary artery disease (Figure 1).

DISCUSSION

This study shows that the LMR was higher in diabetic CAD patients compared with non-diabetic CAD patients. The LMR ratio signifies inflammation and is likely a marker of atherosclerotic burden especially in the presence of multiple atherosclerotic risk factors. Notably, even after adjusting for confounding variables, the association of LMR with CAD remained statistically significant. This observation suggests that LMR may be a marker of higher inflammation along with higher

Table 1 Demographic characteristics and biochemistry parameters of Group I and Group II

Demographic parameters	Group I	Group II	*P* value
Age (yr) (mean ± SD)	54.2 ± 10.2	53.2 ± 10.3	0.473
Gender (male/female)	81/19	89/11	0.82
Diet (non-veg/veg)	81/19	60/40	0.004
Smoker (yes/no)	65/35	50/50	0.022
Alcoholic (yes/no)	26/74	25/75	0.500
Tobacco chewer (yes/no)	49/51	39/61	0.100
Hypertension history (hypertensive/normotensive)	39/61	20/80	0.003
RBS (mg/dL) (mean ± SD)	220.63 ± 100.26	120.61 ± 42.52	0.001
HbA1c (mean ± SD)	8.83 ± 2.03	5.65 ± 0.38	0.001
TC (mg/dL) (mean ± SD)	154.86 ± 51.04	139.54 ± 55.23	0.006
TG (mg/dL) (mean ± SD)	176.03 ± 99.57	124.47 ± 67.05	0.001
HDL (mg/dL) (mean ± SD)	33.82 ± 9.02	35.15 ± 11.28	0.564
LDL (mg/dL) (mean ± SD)	84.74 ± 38.78	80.92 ± 43.55	0.278
VLDL (mg/dL) (mean ± SD)	34.97 ± 21.77	25.50 ± 14.56	0.001
Hb in gm (mean ± SD)	13.49 ± 2.10	13.87 ± 1.90	0.17
WBC in 10^9/L (mean ± SD)	9.92 ± 3.5	10.75 ± 7.5	0.75
Neutrophils, 10^9/L (mean ± SD)	6.25 ± 2.99	6.92 ± 4.85	0.64
Lymphocytes, 10^9/L (mean ± SD)	3.49 ± 1.68	2.46 ± 1.27	0.001
Monocytes, 10^9/L (mean ± SD)	0.58 ± 0.26	0.75 ± 0.66	0.13
Eosinophils, 10^9/L (mean ± SD)	0.27 ± 0.19	0.39 ± 0.59	0.572
LMR (mean ± SD)	6.19 ± 4.14	4.19 ± 2.78	0.001

RBS: Random blood sugar; HbA1c: Glycated hemoglobin; TC: Total cholesterol; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein; Hb: Hemoglobin; WBC: White blood cells; LMR: Lymphocyte to monocyte ratio.

Table 2 Comparison of atherogenic indices between Group I and Group II

Atherogenic index	Group I	Group II	*P* value
Atherogenic index of plasma (mean ± SD)	0.67 ± 0.24	0.51 ± 0.26	0.001
Castelli's risk index I (mean ± SD)	4.88 ± 1.98	4.37 ± 3.42	0.001
Castelli's risk index II (mean ± SD)	2.70 ± 1.50	2.60 ± 2.77	0.115
Atherogenic coefficient (mean ± SD)	3.88 ± 1.98	3.37 ± 3.42	0.001
Non-HDL-C (mean ± SD)	121.27 ± 50.39	104.38 ± 54.59	0.002

AIP: Atherogenic index of plasma; CRI-I: Castelli's Risk Index I; CRI-II: Castelli's Risk Index II; AC: Atherogenic coefficient; NHC: Non-HDL cholesterol.

atherosclerotic burden, ultimately signifying poor prognosis.

CAD is a major cause of mortality and morbidity worldwide and is increasingly becoming a major public health concern in the developing countries[14]. The atherosclerotic plaque formation and progression to CAD is primarily attributed to atherosclerosis, chronic inflammation, and endothelial dysfunction[15]. In the atherosclerotic process the inflammation plays an active role. However, it is unclear that which cell is primarily responsible for the initiation of these cascade processes.

In our study we found that diabetic CAD group had higher frequency of non-vegetarians, smokers and hypertensive individuals. Similar to our data, non-vegetarian diet, smoking, and hypertension have been shown to increase the risk of CAD in diabetes[16-18]. Studies show that elevated lipid levels are prevalent in diabetic CAD patients with high HbA1c levels, and they have more coronary arteries involved in atherosclerosis and often require coronary artery bypass graft

Table 3 Logistic regression analysis of lymphocyte-to-monocyte ratio for diabetic coronary artery disease

Model	Exp(B)	95%CI		*P* value
		Lower	Upper	
LMR (model 1)	1.48	1.28	1.72	0.001
LMR + (model 1 + age, gender, diet, smoking, history of hypertension)	1.49	1.29	1.74	0.001
LMR + (model 2 + high TC, high TG)	1.49	1.27	1.75	0.001
Model 1: Unadjusted				
Model 2: Age + gender + diet + smoking + history of hypertension + model 1				
Model 3: Model 2 + high TC, high TG				

LMR: Lymphocyte-to-monocyte ratio; TC: Total cholesterol, TG: Triglycerides.

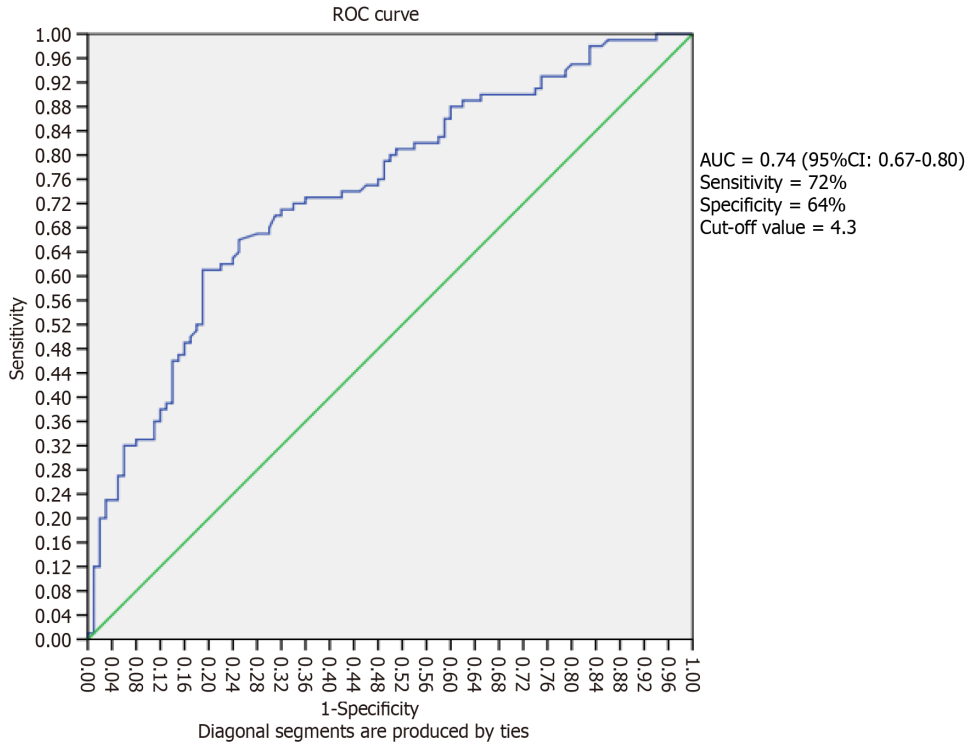

Figure 1 Receiver operating characteristic curve analysis of lymphocyte to monocyte ratio for diabetic coronary artery disease. AUC: Area under the curve; ROC: Receiver operating characteristic.

surgery as a treatment option[19-20]. In our study, we observed similar results which show that the diabetic CAD patients have significantly elevated levels of diabetes specific parameters (blood sugar and HbA1c) and lipid profile (TC, TG, and VLDL).

We observed a significant increase in the risk of diabetic CAD associated with LMR. Importantly, this association remained statistically significant even after adjusting for confounding variables. The results were consistent with previous research including the study by Gong *et al*[12] which showed independent positive association between LMR and severity of coronary artery disease and suggested LMR could be a predictive biomarker for CAD. Hua *et al*[20] revealed that increased levels of subtypes of WBCs were positively associated with high risk of death in CAD. Further, we observed the utility of LMR as a predictor of diabetic coronary artery disease and found LMR cut-off of 4.3 can predict the presence of diabetic coronary artery disease in diabetics with a sensitivity of 74% and specificity of 64%. Several studies have highlighted the significance of LMR (lymphocyte-to-monocyte ratio) as a predictive factor for coronary artery disease. Gong *et al*[12] also reported that LMR value greater than 5.06 could predict atherosclerotic CAD even before angiography. Additionally, Si *et al*[8] identified a LMR value of 4.8 or lower as a novel and independent risk factor for CAD. Furthermore, Kose *et al*[21] suggested that LMR, an easily measurable and cost-effective laboratory parameter, exhibited a significant association with the presence of CAD and high SYNTAX scores in patients with stable angina pectoris. Furthermore, LMR is linked to the process of left ventricle remodeling, the recovery of the myocardium, the buildup of myofibroblasts, and the formation of new blood vessels[22].

Thus, we investigated the pivotal role of the LMR as a prognostic factor in diabetic patients with CAD. We revealed the compelling insights, particularly helping in understanding the prognosis and implications of this ratio in diabetic and non-diabetic CAD patients. It is noteworthy to emphasize that there is paucity of available data for the prognostic significance of the LMR ratio in both diabetic and non-diabetic CAD patients. Nevertheless, our findings underscore a substantial disparity in the LMR ratio between these two groups. This significant difference of LMR ratio between both diabetic and non-diabetic CAD patients highlights the burden of systemic inflammation in CAD patients. The results from the AUC analysis underscore the efficacy of the LMR ratio as a predictive marker in diabetic CAD subgroup. Further, ready availability and easy access for WBC count also adds to its value. The differential patterns observed in the LMR ratio between diabetic and non-diabetic CAD cohorts highlight the interplay between immune responses and CAD pro-gression. Further research upon these findings on larger sample size could provide a deeper understanding of this association and have the way for more targeted therapeutic interventions in such patient populations. Our study provides valuable insight into the association of LMR between diabetic and non-diabetic coronary artery disease. However, we cannot comment on the causality as our study had a cross-sectional design. Future longitudinal studies are required to elucidate the significance and mechanisms resulting in increased LMR in diabetic CAD. Also, the role of LMR as a prognostic marker needs to be determined in prospective studies as it is an easily measured biomarker.

CONCLUSION

This study investigated the role of the LMR among diabetic and non-diabetic coronary artery disease patients. The results emphasize the importance of inflammation, lipid profile, and LMR in the development and progression of CAD in individuals with diabetes. Risk factors such as non-vegetarian diet, smoking, hypertension, elevated triglyceride levels, low HDL-C levels, and high LMR were associated with a higher risk of CAD in diabetic patients. The study also demonstrated that LMR is higher in diabetic CAD group compared with non-diabetic CAD, suggesting its usefulness for risk assessment. These findings have implications for improving CAD management in diabetic patients and call for further research regarding the significance and mechanism of increased LMR in diabetic CAD subgroup.

FOOTNOTES

Author contributions: Dabla PK designed the study; Shrivastav D, Dabla PK, Mehta V analysed the manuscript; Dabla PK provided facilities for biochemical testing and Mehta V and Mehra P provided the facility for the enrolment of patients; all authors reviewed and approved the manuscript.

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Country of origin: India

ORCID number: Pradeep Kumar Dabla 0000-0003-1409-6771.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Early versus delayed necrosectomy in pancreatic necrosis: A population-based cohort study on readmission, healthcare utilization, and in-hospital mortality

Hassam Ali, Faisal Inayat, Vinay Jahagirdar, Fouad Jaber, Arslan Afzal, Pratik Patel, Hamza Tahir, Muhammad Sajeel Anwar, Attiq Ur Rehman, Muhammad Sarfraz, Ahtshamullah Chaudhry, Gul Nawaz, Dushyant Singh Dahiya, Amir H Sohail, Muhammad Aziz

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Hassam Ali, Arslan Afzal, Division of Gastroenterology and Hepatology, East Carolina University Brody School of Medicine, Greenville, NC 27834, United States

Faisal Inayat, Gul Nawaz, Department of Internal Medicine, Allama Iqbal Medical College, Lahore, Punjab 54550, Pakistan

Vinay Jahagirdar, Fouad Jaber, Department of Internal Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, United States

Pratik Patel, Division of Gastroenterology, Mather Hospital and Zucker School of Medicine at Hofstra University, Port Jefferson, NY 11777, United States

Hamza Tahir, Department of Internal Medicine, Jefferson Einstein Hospital, Philadelphia, PA 19141, United States

Muhammad Sajeel Anwar, Department of Internal Medicine, UHS Wilson Medical Center, Johnson City, NY 13790, United States

Attiq Ur Rehman, Muhammad Sarfraz, Division of Gastroenterology and Hepatology, Geisinger Wyoming Valley Medical Center, Wilkes-Barre, PA 18711, United States

Ahtshamullah Chaudhry, Department of Internal Medicine, St. Dominic's Hospital, Jackson, MS 39216, United States

Dushyant Singh Dahiya, Division of Gastroenterology, Hepatology, and Motility, The University of Kansas School of Medicine, Kansas City, KS 66160, United States

Amir H Sohail, Department of Surgery, University of New Mexico School of Medicine, Albuquerque, NM 87106, United States

Muhammad Aziz, Division of Gastroenterology and Hepatology, The University of Toledo, Toledo, OH 43606, United States

Corresponding author: Faisal Inayat, MBBS, Research Scientist, Department of Internal Medicine, Allama Iqbal Medical College, Allama Shabbir Ahmad Usmani Road, Faisal Town,

Lahore, Punjab 54550, Pakistan. faisalinayat@hotmail.com

Abstract

BACKGROUND

Acute necrotizing pancreatitis is a severe and life-threatening condition. It poses a considerable challenge for clinicians due to its complex nature and the high risk of complications. Several minimally invasive and open necrosectomy procedures have been developed. Despite advancements in treatment modalities, the optimal timing to perform necrosectomy lacks consensus.

AIM

To evaluate the impact of necrosectomy timing on patients with pancreatic necrosis in the United States.

METHODS

A national retrospective cohort study was conducted using the 2016-2019 Nationwide Readmissions Database. Patients with non-elective admissions for pancreatic necrosis were identified. The participants were divided into two groups based on the necrosectomy timing: The early group received intervention within 48 hours, whereas the delayed group underwent the procedure after 48 hours. The various intervention techniques included endoscopic, percutaneous, or surgical necrosectomy. The major outcomes of interest were 30-day readmission rates, healthcare utilization, and inpatient mortality.

RESULTS

A total of 1309 patients with pancreatic necrosis were included. After propensity score matching, 349 cases treated with early necrosectomy were matched to 375 controls who received delayed intervention. The early cohort had a 30-day readmission rate of 8.6% compared to 4.8% in the delayed cohort (P = 0.040). Early necrosectomy had lower rates of mechanical ventilation (2.9% *vs* 10.9%, P < 0.001), septic shock (8% *vs* 19.5%, P < 0.001), and in-hospital mortality (1.1% *vs* 4.3%, P = 0.01). Patients in the early intervention group incurred lower healthcare costs, with median total charges of $52202 compared to $147418 in the delayed group. Participants in the early cohort also had a relatively shorter median length of stay (6 *vs* 16 days, P < 0.001). The timing of necrosectomy did not significantly influence the risk of 30-day readmission, with a hazard ratio of 0.56 (95% confidence interval: 0.31-1.02, P = 0.06).

CONCLUSION

Our findings show that early necrosectomy is associated with better clinical outcomes and lower healthcare costs. Delayed intervention does not significantly alter the risk of 30-day readmission.

Key Words: Acute necrotizing pancreatitis; Pancreatic necrosis; Early necrosectomy; Delayed necrosectomy; Readmission, Healthcare costs; Mortality

Core Tip: Clinical evidence regarding the impact of necrosectomy timing on patient outcomes and healthcare costs remains limited. Utilizing propensity-matched cohorts, this nationwide study evaluates the clinical and economic implications of early versus delayed necrosectomy in patients with pancreatic necrosis. Our findings show that early intervention within 48 hours is associated with lower rates of mechanical ventilation, septic shock, and in-hospital mortality. Early necrosectomy also results in substantial cost savings and shorter hospital stays. Intriguingly, the timing of the procedure does not significantly influence the 30-day readmission hazard ratio. These results contribute to the ongoing debate on the optimal timing of necrosectomy, offering evidence-based insights that could improve patient outcomes.

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INTRODUCTION

Acute pancreatitis is a complex and potentially lethal disease. It is a leading cause of gastrointestinal-related hospitalization burden in the United States[1]. Necrosis of the pancreas or peripancreatic tissue can occur in about 20% of patients with acute pancreatitis[2]. Mortality rates can be as high as 20%-30% for patients with infected pancreatic necrotic

collections[3,4]. Patients with necrotizing pancreatitis have historically benefited from surgically removing the necrotic tissue, even in the early phase of the illness[5,6]. It has been suggested that delaying surgery leads to the immune system encapsulating the necrotic pancreatic tissue, making necrosectomy technically easier and possibly decreasing mortality[7, 8]. This hypothesis was validated by a randomized controlled trial from 1997 that showed a delay in surgical intervention beyond the first 12 days lowered mortality, as opposed to intervention within the first 72 hours of admission[9]. The mortality rate for those who underwent the procedure after 12 days dropped from 56% to 27%[9]. Since then, there has been a paradigm shift from surgical procedures to less-invasive endoscopic or percutaneous treatment approaches[10]. The debate over when to intervene has resurfaced as mortality and morbidity have declined due to the recent multidisciplinary nonsurgical management of necrotizing pancreatitis[11-13]. The development of lumen-apposing or large-bore metal stents for endoscopic ultrasound-guided drainage shortened procedure time and resulted in fewer adverse events[14,15]. The available data shows that the optimal timing to perform necrosectomy for patients with pancreatic necrosis is still evolving[16-20].

Despite the recognized clinical importance of pancreatic necrosis, there is a lack of large-scale, data-driven epidemiological studies evaluating the effects of the timing of interventions on clinical outcomes and healthcare expenditures. In this study, we aim to compare the impact of early (within 48 hours) versus delayed (after 48 hours) necrosectomy on 30-day readmission rates, healthcare utilization, and in-hospital mortality in patients with pancreatic necrosis using a large national database from the United States.

MATERIALS AND METHODS

Design and data source

We carried out a retrospective cohort study using the 2016-2019 Nationwide Readmissions Database (NRD) of the Healthcare Cost and Utilization Project[21]. The NRD comprises inpatient admissions and readmissions, accounting for around 60% of all-payer hospitalizations across the United States[21]. Diagnoses and procedures were identified using the International Classification of Diseases, Tenth Revision (ICD-10) codes. The study included patients aged 18 years or older with a non-elective admission for pancreatic necrosis (ICD-10 code K85.x). It was further classified based on patients with necrosectomy within 48 hours (cases) or after 48 hours (controls). The timing of necrosectomy was from admission, and all patients included in the study had a primary diagnosis code for necrotizing pancreatitis. Necrosectomy procedures were endoscopic (ICD-10-PCS 0F9G8), percutaneous (ICD-10-PCS 0F9G3), or surgical (ICD-10-PCS 0F9G0). Exclusions were made for patients admitted in December in order to monitor 30-day readmission rates; there were no necrosectomy or readmissions associated with traumatic injuries. The authors did not use NRD databases before 2016, as the data is coded using ICD-9 codes, which could lead to the misclassification of certain variables. This study report was prepared and revised according to the Strengthening the Reporting of Observational Studies in Epidemiology recommendations[22].

Baseline characteristics

Baseline patient information included demographic variables (age and gender), index admission length of hospital stay and charges, median household income category, primary insurance, discharge outcome, and 30-day readmission status. As in prior studies, the Elixhauser Comorbidity Index was computed to account for multiple comorbidities[23,24].

Outcome measures

The outcomes of interest were early readmission rates (within 30 days of index admission), length of stay, hospital costs, and clinical outcomes, including mechanical ventilation, septic shock, portal venous thrombosis, intensive care unit (ICU) admission, acute kidney injury, new renal replacement therapy during admission, and all-cause inpatient mortality.

Statistical analysis

Little's test was applied to establish if data were missing completely at random (MCAR) with a significance threshold of $P < 0.05$. The variables with over 2% missing data that failed Little's MCAR evaluation underwent multiple imputations (25 datasets) for sensitivity analysis. Data were analyzed employing descriptive statistics for nonparametric databases. Categorical values were reported as percentages, and continuous variables as medians and interquartile ranges. Pearson's chi-square test was utilized to compare categorical variables and the Mann-Whitney test for continuous variables. The impact of the timing of each procedure on outcome variables was evaluated using multivariable regression analysis. The risk of readmission and mortality was ascertained by applying Cox proportional hazard regression analysis. Kaplan-Meier estimates were used to demonstrate differences in 30-day readmission among procedure timings. All statistical analyses were executed using the Statistical Software for Data Science (StataCorp LLC, College Station, TX, United States), version 16.1.

Ethical considerations

The data were acquired from the NRD, a de-identified, publicly accessible registry. This database protects the privacy of patients, physicians, and hospitals. Therefore, the informed consent was waived as the patient identifiers were removed from the hospitalization data. Institutional review board approval was also not required for this study. According to the Healthcare Cost and Utilization Project Data Use Agreement, any individual table cell counts of ≤ 10 have been masked to ensure privacy and compliance. In such instances, data are designated as < 10.

RESULTS

Patient demographics and comorbidities

In the unmatched cohort, 1309 participants met the selection criteria for the study period. Of these, 69 (5.27%) patients were readmitted within 30 days (Table 1). After propensity score matching, 349 cases (patients who underwent necrosectomy within 48 hours) were matched to 375 controls (patients who received necrosectomy after 48 hours). The median age at the time of hospital admission was 55.0 years in both cohorts. With regard to age distribution, the early necrosectomy cohort had more patients aged ≥ 65 years compared to the delayed intervention cohort (29.5% *vs* 21.3%). The distribution of comorbidities among unmatched and matched patients who underwent pancreatic necrosectomy has been outlined (Table 2). There was no significant difference in cardiovascular diseases, pulmonary disorders, diabetes mellitus, renal failure, liver disease, coagulopathy, or obesity in the matched cohorts.

Inpatient outcomes and causes of readmissions

Cases had a lower rate of mechanical ventilation (2.9% *vs* 10.9%, $P < 0.001$), septic shock (8% *vs* 19.5%, $P < 0.001$), ICU admission (0.6% *vs* 99%, $P < 0.001$), acute kidney injury (15.5% *vs* 30.4%, $P < 0.001$), and a lower all-cause inpatient mortality (1.1% *vs* 4.3%, $P = 0.01$) compared to controls (Table 3). The Elixhauser Comorbidity Index score of ≥ 3 was 68.8% in the early cohort compared to 67.2% in the delayed cohort. In the matched cohort, patients who underwent pancreatic necrosectomy within 48 hours had a significantly shorter median length of stay (6 *vs* 16 days, $P < 0.001$) and lower median total hospital charges ($52202 *vs* $147418, $P < 0.001$), compared to those who underwent necrosectomy after 48 hours. There was no increased risk of mortality among matched cohorts, with a hazard ratio (HR) of 0.46 [95% confidence interval (CI): 0.11-1.88, $P = 0.28$].

The 30-day readmission rate was higher for patients who underwent necrosectomy within 48 hours (8.6% *vs* 4.8%, $P = 0.040$) compared to controls. The top five causes of readmission are delineated (Figure 1). The most common cause of readmission in patients who underwent pancreatic necrosectomy within 48 hours was acute pancreatitis with uninfected necrosis (12.5%). In those who underwent pancreatic necrosectomy after 48 hours, it was sepsis due to infection with an unspecified organism (7.1%).

Comparative analysis of necrosectomy timing

Pancreatic necrosectomy timing did not significantly affect the risk of early readmission [HR 0.56 (95%CI: 0.31-1.02), $P = 0.06$]. However, pancreatic necrosectomy after 48 hours had a reduced probability of early readmission over necrosectomy within 48 hours with a log rank of 0.07 (Figure 2).

DISCUSSION

This is the first population-based study to evaluate readmission, healthcare utilization, and in-hospital mortality for early (< 48 hours) versus delayed necrosectomy (> 48 hours) using a national multicenter database. Our findings indicate that early necrosectomy significantly reduces rates of mechanical ventilation, septic shock, and in-hospital mortality. There is a reduced likelihood of readmission after delayed necrosectomy compared to early intervention. However, patients undergoing early intervention have shorter hospital stays and lower inpatient costs.

Hospital readmission constitutes a significant problem in the context of health care policy and reform[25,26]. A number of organizations view readmission rates as a barometer for the quality of healthcare facilities. The body of research on necrotizing pancreatitis-related readmissions has expanded dramatically in recent years. However, there is currently a paucity of clinical evidence comparing the effects of early versus delayed necrosectomy treatments on readmission. In our study, we specifically compared readmission as one of the parameters between early and delayed necrosectomy cohorts. The timing of pancreatic necrosectomy did not significantly affect the risk of early readmission (HR 0.56, $P = 0.06$). However, necrosectomy after 48 hours showed a reduced probability of 30-day readmission over necrosectomy within 48 hours. One possible reason could be the differences in patient characteristics between the matched cohorts. The early necrosectomy cohort had a relatively higher frequency of patients aged 65 years or older compared to the delayed intervention cohort (29.5% *vs* 21.3%). A retrospective analysis of 623 patients who underwent pancreatectomy showed that the patient age of ≥ 65 years independently predicted 30-day unplanned readmissions[27]. Similarly, the Elixhauser Comorbidity Index score of ≥ 3 has been designated as an important variable for readmission prediction in acute pancreatitis[28,29]. In our analysis, the Elixhauser Comorbidity Index score of ≥ 3 was also higher in the early compared to the delayed necrosectomy cohort (68.8% *vs* 67.2%).

The optimal timing to perform necrosectomy for patients with pancreatic necrosis is still evolving[30]. According to the 2020 clinical practice update from the American Gastroenterological Association, the optimal timing for pancreatic debridement should be around four weeks[31]. Early debridement (< 2 weeks after onset) correlates with increased morbidity and mortality[31]. The International Association of Pancreatology/American Pancreatic Association guidelines also suggest that endoscopic treatment for walled-off necrosis be delayed for at least four weeks after the onset of pancreatitis to allow for the encapsulation of necrotic tissue[32]. The delay leads peripancreatic collections to encapsulate, reducing the risk of procedural complications such as bleeding and perforation[33,34]. However, experts recommend early percutaneous drainage for infected or symptomatic necrotic collections[35-39]. Nonetheless, there has been limited investigation into the potential advantages and drawbacks of initiating drainage procedures before the 4-week mark.

Table 1 Clinical characteristics of all patients (index hospitalizations) who underwent pancreatic necrosectomy with a primary diagnosis of pancreatic necrosis, *n* (%)

Factor	Unmatched patients			Matched patients		
	Within 48 hours (cases)	After 48 hours (controls)	*P* value	Within 48 hours (cases)	After 48 hours (controls)	*P* value
Total patients	420	889		349	375	
Length of stay, median (IQR)	6.0 (3.0, 10.0)	23.0 (12.0, 42.0)	< 0.001	6.0 (4.0, 11.0)	16.0 (9.0, 31.0)	< 0.001
Total hospital charges in USD, median (IQR)	51773.0 (28907.0, 92300.0)	196571.0 (89244.0, 408072.0)	< 0.001	52202.0 (29417.0, 101413.0)	147418.0 (73243.5, 316993.0)	< 0.001
30-day readmission	35 (8.3)	34 (3.8)	< 0.001	30 (8.6)	18 (4.8)	0.040
Age in years at admission, median (IQR)	55.0 (41.0, 66.0)	52.0 (39.0, 64.0)	0.080	55.0 (42.0, 66.0)	55.0 (39.0, 64.0)	0.13
Age groups (years)			0.068			0.061
18-34	51 (12.1)	144 (16.2)		38 (10.9)	57 (15.2)	
35-49	113 (26.9)	241 (27.1)		91 (26.1)	100 (26.7)	
50-64	134 (31.9)	301 (33.9)		117 (33.5)	138 (36.8)	
≥ 65	122 (29.0)	203 (22.8)		103 (29.5)	80 (21.3)	
Elixhauser comorbidity index score			< 0.001			0.32
0	32 (7.6)	18 (2.0)		20 (5.7)	18 (4.8)	
1	37 (8.8)	46 (5.2)		24 (6.9)	40 (10.7)	
2	71 (16.9)	91 (10.2)		65 (18.6)	65 (17.3)	
≥ 3	280 (66.7)	734 (82.6)		240 (68.8)	252 (67.2)	
Primary payer			0.65			0.37
Medicare	129 (33.9)	260 (30.3)		112 (35.8)	109 (30.4)	
Medicaid	73 (19.2)	166 (19.4)		57 (18.2)	70 (19.6)	
Private	158 (41.5)	381 (44.5)		127 (40.6)	151 (42.2)	
Other	21 (5.5)	50 (5.8)		17 (5.4)	28 (7.8)	
Median household income national quartile for patient ZIP Code			0.28			0.53
1st (0-25th)	105 (25.5)	197 (22.3)		88 (25.7)	88 (23.6)	
2nd (26th-50th)	114 (27.7)	256 (28.9)		94 (27.4)	107 (28.7)	
3rd (51st-75th)	109 (26.5)	271 (30.6)		87 (25.4)	109 (29.2)	
4th (76th-100th)	84 (20.4)	161 (18.2)		74 (21.6)	69 (18.5)	
Disposition of patient (uniform)			< 0.001			0.12
Routine	264 (62.9)	396 (44.6)		211 (60.5)	214 (57.2)	
Transfer to SNF, STH, ICF, and another facility, or AMA	57 (13.6)	182 (20.5)		47 (13.5)	60 (16.0)	
HHC	94 (22.4)	259 (29.2)		87 (24.9)	84 (22.5)	

STH: short-term hospital; SNF: Skilled nursing facility; ICF: Intermediate care facility; HHC: Home health care; AMA: Against medical advice.

The effects of necrosectomy timing on healthcare resource utilization have not been well characterized. Our study revealed that patients in the delayed necrosectomy cohort had greater healthcare utilization and costs. It could be attributed to higher requirements for mechanical ventilation, a greater incidence of septic shock, ICU admission, and acute kidney injury associated with delayed necrosectomy (> 48 hours). Systemic inflammatory response syndrome, commonly seen in severe acute pancreatitis, can lead to multiorgan failure[40,41]. Therefore, delayed intervention may increase the risk of clinical deterioration due to infections and acute inflammatory responses, which can ultimately result in organ failure. It could lead to longer hospitalizations and higher inpatient costs in the delayed intervention group. Contrarily, a recent meta-analysis concluded that patients with early intervention were more likely to have mortality, organ failure, larger fluid collections, and less encapsulation[42]. However, it is possible that the retrospective nature of

Table 2 Distribution of Elixhauser comorbidities among all patients (index hospitalizations) who underwent pancreatic necrosectomy with a primary diagnosis of pancreatic necrosis, *n* (%)

Factor	Unmatched patients			Matched patients		
	Within 48 hours (cases)	After 48 hours (controls)	*P* value	Within 48 hours (cases)	After 48 hours (controls)	*P* value
Total patients	420	889		349	375	
Congestive heart failure	35 (8.3)	81 (9.1)	0.64	30 (8.6)	29 (7.7)	0.67
Cardiac arrhythmias	55 (13.1)	287 (32.3)	< 0.001	48 (13.8)	58 (15.5)	0.51
Uncomplicated hypertension	230 (54.8)	458 (51.5)	0.27	194 (55.6)	200 (53.3)	0.54
Chronic pulmonary diseases	81 (19.3)	135 (15.2)	0.062	72 (20.6)	59 (15.7)	0.087
Uncomplicated diabetes	70 (16.7)	119 (13.4)	0.11	58 (16.6)	53 (14.1)	0.35
Complicated diabetes	66 (15.7)	170 (19.1)	0.13	55 (15.8)	64 (17.1)	0.64
Hypothyroidism	19 (4.5)	83 (9.3)	0.002	17 (4.9)	27 (7.2)	0.19
Renal failure	29 (6.9)	66 (7.4)	0.74	24 (6.9)	25 (6.7)	0.91
Liver disease	69 (16.4)	227 (25.5)	< 0.001	54 (15.5)	55 (14.7)	0.76
Coagulopathy	31 (7.4)	136 (15.3)	< 0.001	20 (5.7)	25 (6.7)	0.60
Obesity	60 (14.3)	204 (22.9)	< 0.001	43 (12.3)	43 (11.5)	0.72
Weight loss	118 (28.1)	444 (49.9)	< 0.001	108 (30.9)	124 (33.1)	0.54
Fluid and electrolyte disorder	227 (54.0)	634 (71.3)	<0.001	215 (61.6)	211 (56.3)	0.14
Iron-deficiency anemia	33 (7.9)	67 (7.5)	0.84	29 (8.3)	24 (6.4)	0.32
Alcohol abuse	110 (26.2)	271 (30.5)	0.11	97 (27.8)	108 (28.8)	0.76
Drug abuse	21 (5.0)	78 (8.8)	0.016	15 (4.3)	26 (6.9)	0.13
Depression	72 (17.1)	163 (18.3)	0.60	59 (16.9)	73 (19.5)	0.37
Complicated hypertension	32 (7.6)	86 (9.7)	0.23	26 (7.4)	27 (7.2)	0.90

the studies included in the meta-analysis could have caused a between-group imbalance in patient profiles[42]. Consequently, the increased occurrence of organ failure in the early intervention group might have adversely influenced clinical outcomes[42]. In our cohorts, we could not stratify the individuals according to the specific etiologies of pancreatitis due to inconsistent data availability in the NRD database. Certain etiologies, like post-endoscopic retrograde cholangiopancreatography and autoimmune pancreatitis, may lead to more severe hospital courses. This observation indicates that such etiologies may have an impact on clinical outcomes such as length of stay and mortality. Therefore, future studies should aim to include etiological factors to better understand their influence on the disease course and outcomes.

Our findings show a mortality benefit associated with early compared to delayed necrosectomy (1.1% *vs* 4.3%, $P = 0.01$). It could be related to the relatively quick resolution of pancreatic necrosis, which led to a lower rate of complications. An Indian randomized control trial showed that patients with infected necrotizing pancreatitis had a significantly higher and faster resolution of organ failure with proactive percutaneous catheter drainage[43]. A meta-analysis of nine studies based on pooled data from 870 patients showed that there was no significant difference in mortality and complications between early and delayed minimally invasive intervention groups, denoting early intervention as safe for infected pancreatic necrosis[44]. A meta-analysis of four studies analyzing the data of 427 patients who underwent endoscopic treatment also showed no significant difference in rates of mortality and adverse events in early versus late groups[45]. Similarly, a meta-analysis of seven studies also showed no significant mortality or new-onset organ failure difference between early and delayed groups[46]. Therefore, early minimally invasive procedures do not have a negative impact on patient outcomes but may possibly lead to longer hospital and ICU stays[46]. A meta-analysis of six studies based on 630 patients revealed no statistically significant differences in overall adverse events or mortality, but early drainage may prolong the length of stay compared to standard endoscopic ultrasound-guided drainage[47]. Our cohort study revealed a mortality benefit, a shorter hospital stay, and a reduced need for ICU admission in the early group compared to the delayed group.

Sepsis was the most common cause of 30-day readmission in patients who underwent delayed necrosectomy. It indicates that more patients in our delayed necrosectomy cohort might have developed infected necrosis. It could also expose these patients to a number of inpatient complications. A retrospective study revealed that postponing necrosectomy until 30 days after index hospitalization had some mortality benefit, but it was associated with longer use

Table 3 Clinical outcomes of all patients (index hospitalizations) who underwent pancreatic necrosectomy with a primary diagnosis of pancreatic necrosis, *n* (%)

Factor	Unmatched patients			Matched patients		
	Within 48 hours (cases)	After 48 hours (controls)	*P* value	Within 48 hours (cases)	After 48 hours (controls)	*P* value
Total patients	420	889		349	375	
Mechanical ventilation	14 (3.3)	155 (17.4)	< 0.001	< 10	41 (10.9)	< 0.001
Septic shock	38 (9.0)	239 (26.9)	< 0.001	28 (8.0)	73 (19.5)	< 0.001
Portal venous thrombosis	35 (8.3)	71 (8.0)	0.83	30 (8.6)	27 (7.2)	0.49
ICU-level admission	< 10	145 (16.3)	< 0.001	< 10	37 (9.9)	< 0.001
Acute kidney injury	64 (15.2)	330 (37.1)	< 0.001	54 (15.5)	114 (30.4)	< 0.001
New RRT during admission	15 (3.6)	43 (4.8)	0.30	13 (3.7)	< 10	0.20
Died during hospitalization	< 10	50 (5.6)	< 0.001	< 10	16 (4.3)	0.010

ICU: Intensive care unit; RRT: Renal replacement therapy. NOTE: According to the Healthcare Cost and Utilization Project Data Use Agreement, any individual table cell counts of ≤ 10 have been masked to ensure privacy and compliance. In such instances, data are designated as < 10.

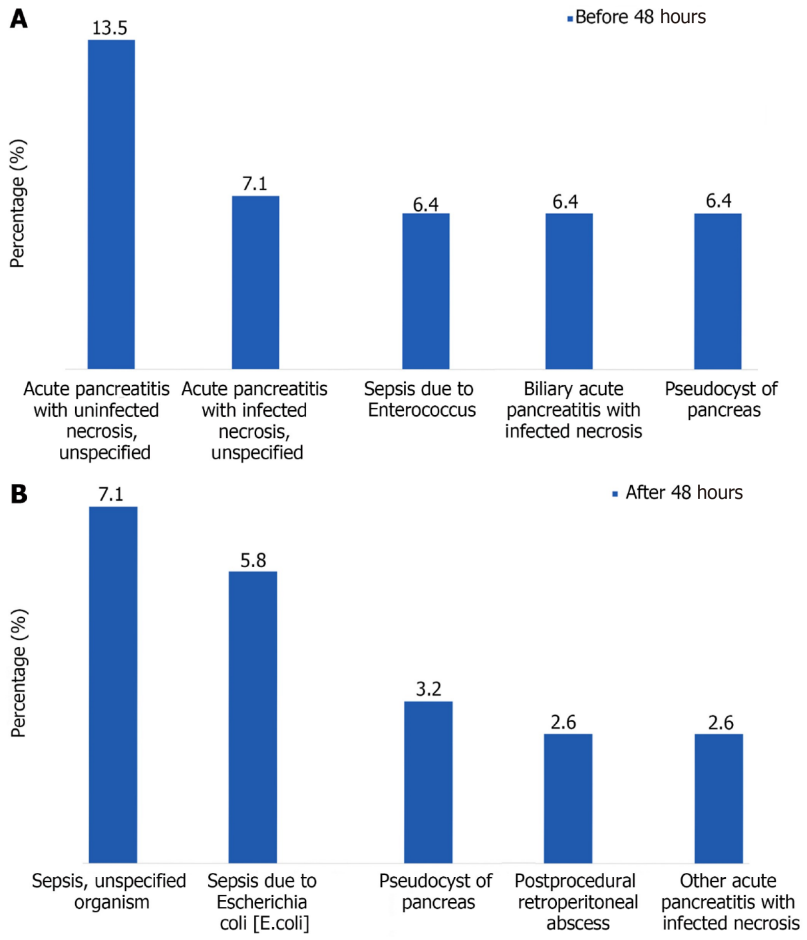

Figure 1 Absolute rates of cause-specific 30-day readmission stratified by pancreatic necrosectomy timing on index admission in the matched cohort. A: Before 48 hours; B: After 48 hours.

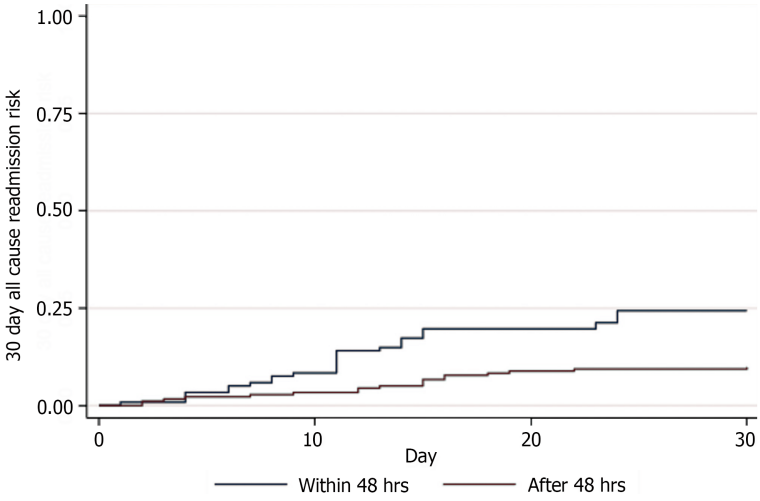

Figure 2 The 30-day readmission risk based on pancreatic necrosectomy timing on index acute pancreatitis admission in the matched cohort (log rank = 0.07).

of antibiotics and an increased occurrence of infections with Candida species and drug-resistant bacteria[48]. In patients with infected necrosis, Gram-negative infections are frequent, but Gram-positive enterococci and fungi have also been reported[49]. Nonetheless, several randomized controlled trials have revealed that empiric broad-spectrum antibiotics do not influence the likelihood of developing infected necrosis, multiorgan complications, mortality, or surgery in patients with severe acute necrotizing pancreatitis[50-52]. A randomized controlled trial from the United Kingdom advocated for procalcitonin-directed care to lower the administration of antibiotics in patients with acute pancreatitis[53]. Similarly, a retrospective study from China underscored the value of early prognostication of acute pancreatitis based on etiology and disease severity for antibiotic use[54]. Currently, there is insufficient evidence for routine prophylactic use of antifungals in these patients. The existing clinical data also show marked heterogeneity in terms of non-interventional supportive care[55]. Therefore, supportive treatment during the periprocedural period for pancreatic necrosis merits further research [55].

The participants in our study received endoscopic, percutaneous, or surgical necrosectomy procedures. A recent nationwide study of 4605 patients with infected necrosis showed that open debridement had a significantly higher risk of mortality, respiratory failure, mechanical ventilation, and acute kidney injury compared to minimally invasive procedures[56]. In our delayed cohort, patients experienced similar complications and had a higher mortality risk compared to the early cohort. We could not stratify patients based on the respective necrosectomy procedures they received due to NRD data limitations. However, it is plausible that a significant number of patients in our delayed cohort received surgical necrosectomy. A retrospective cohort study from Australia showed that delayed surgical intervention alone may have higher odds of additional complications such as pancreatic fistulae and new-onset diabetes mellitus compared to endoscopic and percutaneous approaches[57]. Furthermore, a retrospective study from the United States showed that endoscopic necrosectomy resulted in reduced morality risk, complications, hospital stay, and inpatient charges compared to percutaneous and surgical procedures[58]. A network meta-analysis of seven studies using pooled data from 400 patients designated the step-up approach with endoscopic debridement as the first choice for infected pancreatic necrosis[59]. Moreover, it was argued that surgical debridement (early and delayed) should be avoided[59]. A meta-analysis of 10 studies based on 570 patients revealed the delayed surgical step-up approach as the optimal choice for acute necrotizing pancreatitis and advocated avoiding drainage alone[60].

Current clinical evidence indicates a regimental shift towards a step-up approach in managing patients with pancreatic necrosis[61-63]. The step-up approach described in a trial from the Netherlands showed better outcomes regarding major complications and mortality than primary open necrosectomy[64]. Similarly, a trial conducted in the United States found no notable discrepancy in mortality rates but a significantly higher rate of complications in the surgical group (pancreatocutaneous fistula) compared to the endoscopic step-up approach[65]. A multicenter trial showed that upfront necrosectomy at the index intervention rather than as a step-up procedure may safely reduce the number of reinterventions in stable patients with fully encapsulated collections and infected necrotizing pancreatitis[66]. However, a cohort study from Germany found that an endoscopic step-up approach reduced peri-interventional morbidity and length of hospital stay[67]. Therefore, further research is warranted to evaluate the best therapeutic strategy utilizing novel technological advancements for patients with pancreatic necrosis[68,69].

This cohort study has several strengths. It has a large sample population, sourced from one of the largest all-payer data sets in the United States. This specific characteristic distinguishes our research from previous studies by providing a reasonable degree of generalizability about the outcomes of early versus delayed necrosectomy. It broadens the applicability of the results in clinical practice compared to single-center experiences with more restricted information on the subject. Using a robust analytical approach, we found that delayed necrosectomy (> 48 hours) is associated with significantly prolonged hospitalization and increased healthcare charges. It offers pertinent real-world insights and clinical evidence to gastroenterologists and gastrointestinal surgeons regarding necrosectomy timing. Therefore, it may aid in informed therapeutic decision-making and prognostic advice. Our results may serve to enable pancreatologists to

conduct future studies expanding data on the risks and complications associated with early intervention compared to delayed strategies. It may help to refine patient selection criteria for early necrosectomy, potentially reducing postprocedure adverse clinical outcomes[70]. Further research is warranted to investigate the long-term impact of early versus delayed necrosectomy.

Limitations

There are certain limitations to our study. The retrospective cohort nature of our design renders it susceptible to the biases commonly associated with such studies. Furthermore, the NRD database lacks specific information on factors such as the severity of acute pancreatitis, the course of hospitalization, treatment modalities, and time intervals related to complications. The database specifies the interval between hospital admission and the necrosectomy procedure. However, it does not include patient data about the interval between hospital admission and the diagnosis of pancreatic necrosis. Our analysis did not account for the specific etiologies of pancreatitis, representing a potential limitation of our findings. We also could not stratify clinical outcomes by specific necrosectomy techniques or individual patients undergoing multiple procedures. Due to the lack of granular data in the database, this study could not specifically track the number or percentage of readmissions due to postprocedure complications. Finally, it is crucial to recognize that human coding errors may occur in the NRD because it is an administrative database reliant on ICD codes for data storage. Despite these constraints, this is the first study to compare patient outcomes between early and late necrosectomy procedures using a nationwide database. It will improve the paucity of data regarding the timing of intervention for pancreatic necrosis.

CONCLUSION

Our study showed that early necrosectomy was associated with improved clinical outcomes, including decreased risks of septic shock, mechanical ventilation, ICU admission, acute kidney injury, and lower all-cause inpatient mortality. Patients in the early cohort had relatively shorter hospital stays and less expensive medical care. The 30-day readmission rate was higher for patients who underwent early necrosectomy within 48 hours compared to those who received delayed intervention after 48 hours. As the management of necrotizing pancreatitis is continually evolving, our analysis shows that early necrosectomy may have certain clinical benefits over delayed intervention. Therefore, further research is required to stratify the long-term impact of various early interventions on patients with pancreatic necrosis.

FOOTNOTES

Author contributions: Ali H, Inayat F, Jahagirdar V, Jaber F, Afzal A, Patel P, and Tahir M concepted and designed the study, participated in the acquisition of data, interpretation of results, writing of the original draft, and critical revisions of the important intellectual content of the final manuscript; Anwar MS, Rehman AU, Sarfraz M, Chaudhry A, Nawaz G, Dahiya DS, and Sohail AH contributed to the analysis and interpretation of results and drafting of the manuscript; Aziz A reviewed, revised, and improved the manuscript by suggesting pertinent modifications; and all authors critically assessed, edited, and approved the final manuscript and are accountable for all aspects of the work.

Institutional review board statement: The data of patients was not acquired from any specific institution but rather open-access United States National Readmission Database (NRD) database. The NRD contains de-identified information, protecting the privacy of patients, physicians, and hospitals. Therefore, this study was deemed exempt from the institutional review board.

Informed consent statement: Participants were not required to give informed consent for this retrospective cohort study since the analysis of baseline characteristics used anonymized clinical data.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article. The preliminary form of these data was presented as an abstract at the Digestive Disease Week (DDW), May 19-21, 2024 in Washington, DC, United States.

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Country of origin: United States

ORCID number: Hassam Ali 0000-0001-5546-9197; Faisal Inayat 0000-0001-7576-7319; Vinay Jahagirdar 0000-0001-6685-1033; Pratik Patel 0000-0003-1375-8542; Dushyant Singh Dahiya 0000-0002-8544-9039.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Semaglutide for the management of diabesity: The real-world experience

Mohammed Alkhalifah, Hafsa Afsar, Anindya Shams, Dania Blaibel, Vishnu Chandrabalan, Joseph M Pappachan

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Mohammed Alkhalifah, Hafsa Afsar, Anindya Shams, Dania Blaibel, Joseph M Pappachan, Department of Endocrinology and Metabolism, Lancashire Teaching Hospitals NHS Trust, Preston PR2 9HT, United Kingdom

Mohammed Alkhalifah, Department of Family Medicine, King Faisal Specialist Hospital, Riyadh 11211, Saudi Arabia

Vishnu Chandrabalan, Department of Data Science, Lancashire Teaching Hospitals NHS Trust, Preston PR2 9HT, United Kingdom

Joseph M Pappachan, Faculty of Science, Manchester Metropolitan University, Manchester M15 6BH, United Kingdom

Joseph M Pappachan, Faculty of Biology, Medicine & Health, The University of Manchester, Manchester M13 9PL, United Kingdom

Corresponding author: Joseph M Pappachan, MD, FRCP, Academic Editor, Consultant Endocrinologist, Professor, Department of Endocrinology and Metabolism, Lancashire Teaching Hospitals NHS Trust, Sharoe Green Lane, Preston PR2 9HT, United Kingdom.
drpappachan@yahoo.co.in

Abstract

BACKGROUND

Diabesity (diabetes as a consequence of obesity) has emerged as a huge healthcare challenge across the globe due to the obesity pandemic. Judicious use of anti-diabetic medications including semaglutide is important for optimal management of diabesity as proven by multiple randomized controlled trials. However, more real-world data is needed to further improve the clinical practice.

AIM

To study the real-world benefits and side effects of using semaglutide to manage patients with diabesity.

METHODS

We evaluated the efficacy and safety of semaglutide use in managing patients with diabesity in a large academic hospital in the United States. Several parameters were analyzed including demographic information, the data on impro-

vement of glycated hemoglobin (HbA1c), body weight reduction and insulin dose adjustments at 6 and 12 months, as well as at the latest follow up period. The data was obtained from the electronic patient records between January 2019 to May 2023.

RESULTS

106 patients (56 males) with type 2 diabetes mellitus (T2DM), mean age 60.8 ± 11.2 years, mean durations of T2DM 12.4 ± 7.2 years and mean semaglutide treatment for 2.6 ± 1.1 years were included. Semaglutide treatment was associated with significant improvement in diabesity outcomes such as mean weight reductions from baseline 110.4 ± 24.6 kg to 99.9 ± 24.9 kg at 12 months and 96.8 ± 22.9 kg at latest follow up and HbA1c improvement from baseline of 82 ± 21 mmol/mol to 67 ± 20 at 12 months and 71 ± 23 mmol/mol at the latest follow up. An insulin dose reduction from mean baseline of 95 ± 74 units to 76.5 ± 56.2 units was also observed at the latest follow up. Side effects were mild and mainly gastrointestinal like bloating and nausea improving with prolonged use of semaglutide.

CONCLUSION

Semaglutide treatment is associated with significant improvement in diabesity outcomes such as reduction in body weight, HbA1c and insulin doses without major adverse effects. Reviews of largescale real-world data are expected to inform better clinical practice decision making to improve the care of patients with diabesity.

Key Words: Type 2 diabetes mellitus; Diabesity; Glucagon-like peptide 1 receptor agonists; Semaglutide; Insulin dose reduction; Weight loss

Core Tip: Rational medical management of diabesity, *i.e.*, diabetes resulting from obesity, involves judicious use of antidiabetic drugs which should ideally help body weight loss while controlling hyperglycemia. Although semaglutide use has been associated with significant improvements in body weight and glycated hemoglobin (HbA1c) in multiple randomized controlled trials (RCTs) and prospective observational studies, more real-world data from day-to-day medical practice would inform better clinical decision making. We report our retrospective study data that reveals better diabesity outcomes compared to RCTs with a mean weight loss of 12.3%, HbA1c reduction of 13.7% and insulin dose reduction of 19.5% with semaglutide treatment.

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INTRODUCTION

The global obesity pandemic in the past few decades has resulted in a substantial increase in the prevalence of patients with type 2 diabetes mellitus (T2DM) across the world. According to the International Diabetes Federation (IDF) estimates, there were 537 million adults worldwide living with diabetes in the year 2021, the majority of whom were suffering from T2DM[1]. In a significant proportion of patients with T2DM, diabetes occurs as a direct consequence of obesity or abdominal adiposity. Diabesity is an important concept to denote the strong pathobiological interlink between obesity and T2DM, which has important therapeutic implications as control of both diseases becomes imperative in the optimal care of these patients[2]. However, glycemic management is often given priority even by diabetologists while managing diabesity, which can potentially worsen obesity as several of the antidiabetic medications including insulins may cause weight gain. Moreover, obesity is usually a progressive disease in many individuals, and therefore, diabesity is very likely to worsen over time unless the appropriate management strategies are adopted early in the course of illness by managing obesity with lifestyle and pharmacological interventions.

The novel antidiabetic medications belonging to the glucagon like peptide-1 receptor agonist (GLP-1RA) class were available for managing diabesity over more than one and a half decades, and newer agents are currently being introduced into the global market, some of which are also used for weight management even in patients without T2DM. This class of drugs acts through pancreatic and extra-pancreatic mechanisms causing meal-related pancreatic insulin secretion and suppression of endogenous glucagon, appetite suppression, and early satiety by central and peripheral mechanisms (by delaying gastrointestinal nutrient transit), and with a strong tendency for body weight reduction[2,3]. Semaglutide is one of the latest additions to the list of GLP-1RA molecules and has been in use for over the past 6 years in many countries including the United Kingdom (UK). The Semaglutide Unabated Sustainability in Treatment of Type 2 Diabetes (SUSTAIN) Trials, and the Peptide Innovation for Early Diabetes Treatment (PIONEER) Trials tested the efficacy

of semaglutide (1 mg) subcutaneously weekly for the management of T2DM, while Semaglutide Treatment Effect in People with Obesity (STEP) Trial tested the efficacy of the molecule (at 2.4 mg weekly dose) for body weight management [4]. These large multinational randomized controlled clinical trials (RCTs), viz., SUSTAIN 1-11, PIONEER 1-12 and STEP 1-6 trials, demonstrated the efficacy and safety of semaglutide as an antidiabetic medication with good weight loss potential beyond doubt.

As the settings of RCTs and prospective observational studies are well-supervised and often rigorously scrutinized by research teams, the study results captured by these methods may not always reflect the actual real-world clinical picture in our day-to-day medical practice. Therefore, it is imperative to have real-world clinical data for truly appraising the actual immediate and long-term efficacy and safety of semaglutide to aid therapeutic decision making for patients with diabesity and T2DM. The present study is such an attempt to gather the real-world data from patients managed on long-term basis with semaglutide for diabesity in a large academic teaching hospital in the UK.

MATERIALS AND METHODS

Study design and settings

This is a retrospective clinical study by review of the clinical and therapeutic data of all patients managed with any one of the injectable (brand: Ozempic), or oral (brand: Rybelsus) semaglutide agents, between January 1, 2019 and May 31, 2023 at Lancashire Teaching Hospitals NHS Trust (LTHTR). The endocrine and metabolic service of this hospital provides comprehensive diabetic care for patients in the Central Lancashire and South Cumbria regions of the UK, with a population of about 0.4 million people. The study was approved by the institutional audit/research committee (No: DIAB/CA/2022-23-08).

Participants

All adult patients (aged ≥ 18 years) with T2DM treated by one of the above GLP-1RA molecules for management of their diabetes were considered for inclusion in the study.

Data capture

Electronic medical records were searched for all patients with a diagnosis of diabetes mellitus treated with semaglutide during the study period. The total number of cases in this category were further reviewed for inclusion in the study.

Inclusion criteria

(1) Patients with a diagnosis of T2DM managed with injectable or oral semaglutide; and (2) Participants with predefined primary outcome measures (HbA1c alteration from baseline values to different follow-up periods) and/or secondary outcomes such as alterations in body weight, as well as reduction in insulin dose – all these outcomes with meaningful data in at least one of the follow-up periods (6 months, 12 months and/or at the last follow up just prior to completion of the study). Additional factors examined were blood pressure, renal functions, and urine albumin creatinine ratio.

Exclusion criteria

(1) Patients with a diagnosis of type 1 diabetes mellitus; (2) Incomplete study data to obtain meaningful outcome measures as specified above; and (3) Follow-up duration less than 6 months.

Data collection

Data was collected by reviewing each case fulfilling the inclusion criteria identified from the total number of cases in the electronic patient record system (Flex) of the LTHTR. Microsoft Excel spreadsheet was used for data compilation and rechecked for logical inconsistency and entry error. Data was cleaned accordingly before analysis.

Data analysis

Data management and statistical analysis were done using statistical package for social sciences (SPSS software Version 26). The analysis included frequency distribution, cross-tabulation as well as descriptive statistical analysis. Paired t-test was performed to ascertain statistical significance of change in glycemic status, weight, and dose of insulin before and after semaglutide therapy. The results were presented in tables and graphs.

RESULTS

A total of 106 patients with T2DM on semaglutide were included in this study. 56 were males and the remainder females. Table 1 shows their baseline clinical and biochemical characteristics. The data analysis has been done separately for male and female patients for each of the variables. The mean (SD) duration of diabetes was 12.4 (7.2) years, and the mean follow up duration after initiation of semaglutide was 2.6 (1.1) years, and the Table 2 shows other different antidiabetic medications received by patients prior to the initiation of semaglutide. Among different anti-diabetic drugs, 69.8% patients were on metformin, 69.8% were on insulin and 51.9% were on sodium glucose cotransporter 2 (SGLT2) inhibitors.

Table 1 Baseline characteristics of 106 type 2 diabetes mellitus patients treated with semaglutide

Attributes	Male		Female		All		Reference value (if applicable)
	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Patient age (years)	60.88	(10.04)	60.64	(12.43)	60.76	(11.18)	
Systolic blood pressure (mm Hg)	140.43	(18.42)	136.79	(15.18)	138.86	(17.02)	
Diastolic blood pressure (mm Hg)	80.32	(10.82)	71.32	(10.62)	76.45	(11.56)	
Patient baseline weight (kg)	111.45	(21.07)	109.05	(28.63)	110.36	(24.56)	
Patient baseline HbA1c (mmol/mol)	84.18	(22.20)	79.46	(19.40)	81.96	(20.96)	20-41 mmol/mol
Serum creatinine (μmol/L)	101.16	(53.98)	75.33	(37.18)	89.34	(48.52)	Male: 59-104 μmol/L; Female: 45-84 μmol/L
Urine ACR (mg/mmol)	21.96	(38.26)	28.74	(58.59)	24.97	(47.81)	Male: < 2.5 mg/mmol; Female: < 3.5 mg/mmol
Total cholesterol (mmol/L)	4.10	(1.47)	4.53	(1.16)	4.29	(1.35)	< 5.01 mmol/L
HDL (mmol/L)	1.07	(0.34)	1.16	(0.36)	1.11	(0.35)	> 1 mmol/L
Triglyceride (mmol/L)	4.82	(5.79)	5.02	(2.62)	4.89	(4.60)	< 2.3 mmol/L
ALT (U/L)	25.50	(15.04)	23.73	(11.17)	24.69	(13.33)	0-33 U/L

ALT: Alanine transaminase; ACR: Albumin creatinine ratio; HbA1c: Glycated hemoglobin; HDL: High-density lipoprotein.

Table 2 Treatment patients received for type 2 diabetes mellitus before initiation of semaglutide therapy

Medication	Total patients	Percentage (%)
Metformin	74	69.8
SGLT2 inhibitors	55	51.9
Sulfonylureas	27	25.5
Pioglitazone	7	6.6
Meglitinides	1	0.9
DPP4 inhibitors	6	5.7
Insulin	74	69.8
Anti-obesity medications	1	0.9

98 (92.5%) patients were on subcutaneous semaglutide while the remaining 8 were on the oral drug. Eight (7.5%) patients experienced gastrointestinal side effects (mainly nausea and bloating) after initiation of semaglutide but could tolerate the drug later (4 patients had to stop the drug within 3 months of initiation of semaglutide and have not been included in the analysis).

Table 3 focuses on the baseline HbA1c and its changes over various time intervals after initiating semaglutide. Baseline mean HbA1c was 81.96 (SD 20.96) mmol/mol which reduced to 66.91 (SD 19.88) mmol/mol after one year. Figure 1 displays the HbA1c reduction trend.

Table 4 shows the paired *t*-test for HbA1c, and compares HbA1c at baseline to 6 months, 1 year and at the latest follow-up separately. *P* value was < 0.05 in each of the scenarios.

Table 5 displays the baseline body weight and its changes over time after initiating semaglutide.

Although the mean weight reduction from baseline to follow-up at 6 months and 12 months did not show statistical significance, weight loss at the latest follow-up period showed a tendency to reach significance ($P = 0.057$). The mean weight reduction was 12.3% from baseline. The weight loss tendency plateaued between 6-12 months. The mean decline in weight during the first 6 months was more profound than the decline which happened after 1 year to the latest follow-up. The weight reduction trend is shown in Figure 2.

Table 6 displays daily insulin requirements in the patients before and after starting semaglutide. We noted that the baseline insulin dose was lower in the females compared to males. Additionally, the dose reductions in units were greater among females compared to males. Four patients could completely stop insulin after treatment with semaglutide. The total insulin dose reduction from baseline to last follow-up was statistically significant ($P = 0.024$) (Figure 3).

Table 3 Patients' glycated hemoglobin before and after treatment with semaglutide

Attributes	Male		Female		All		Reference value (if applicable)
	Mean	(SD)	Mean	(SD)	Mean	(SD)	
Patient baseline HbA1c (mmol/mol)	84.18	(22.20)	79.46	(19.40)	81.96	(20.96)	20-41 mmol/mol
HbA1c after 6 months (mmol/mol)	68.52	(21.06)	67.02	(15.66)	67.79	(18.53)	20-41 mmol/mol
HbA1c after 1 yr (mmol/mol)	71.42	(21.94)	63.27	(17.48)	66.91	(19.88)	20-41 mmol/mol
Latest HbA1c (mmol/mol)	72.98	(25.60)	68.2	(19.80)	70.73	(23.04)	20-41 mmol/mol

HbA1c: Glycated hemoglobin.

Table 4 Effect of semaglutide on glycemic control (glycated hemoglobin)

HbA1c	Mean	SD	*df*	Sig. (2-tailed) *P* value
HbA1c baseline to 6 month	12.31	17.926	47	0.000
HbA1c baseline to 1 yr	14.456	19.428	47	0.000
HbA1c baseline to latest	9.644	20.174	47	0.002

HbA1c: Glycated hemoglobin.

Table 5 Patient body weight before and after treatment with semaglutide

Attributes	Male		Female		All	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Patient baseline weight (kg)	111.45	(21.07)	109.05	(28.63)	110.36	(24.56)
Weight after 6 months (kg)	100.19	(20.98)	100.39	(38.57)	100.27	(29.13)
Weight after 1 year (kg)	102.10	(25.96)	98.07	(25.35)	99.86	(24.94)
Latest weight (kg)	101.74	(18.21)	90.21	(22.93)	96.82	(22.88)

Table 6 Daily insulin requirement before and after treatment with semaglutide

Attributes	Male		Female		All	
	Mean	(SD)	Mean	(SD)	Mean	(SD)
Daily insulin dose baseline (units)	106.00	(83.59)	81.89	(58.48)	95.02	(74.02)
Daily insulin dose latest (units)	92.2	(62.86)	62.14	(46.21)	76.45	(56.17)

DISCUSSION

Several previous studies including multiple RCTs, and observational studies illustrate consistent beneficial effects of semaglutide therapy particularly in patients with diabesity. Our data revealed significant improvements in diabesity outcomes such as a mean weight reduction of 12.3% and a mean HbA1c reduction of 13.7% from baseline at the latest follow up period at a mean follow up duration of 2.6 ± 1.1 years. A mean 19.5% reduction of total insulin dose was possible in those patients who were already established on insulin management for longstanding inadequately controlled T2DM, and 4 patients could even completely stop insulin. This real-world data reinforces our understanding on the therapeutic benefits of this new GLP-1RA molecule, semaglutide, for optimal management of diabesity in day-to-day medical practice. Unfortunately, we couldn't procure enough data in this audit sufficiently powered to analyze the cardiovascular and metabolic implications of semaglutide treatment as observed in major RCTs. However, we observed remarkably great improvements in diabesity outcomes such as HbA1c reduction and weight loss compared to those observed in large RCTs.

A recent systematic review of major clinical trials showed a mean HbA1c reduction of 0.97% (95%CI: -1.33 to -0.62) and 1.36% (95%CI: -1.59 to -1.13) with 0.5 mg and 1.0 mg of semaglutide respectively as subcutaneous (S/c) weekly injections

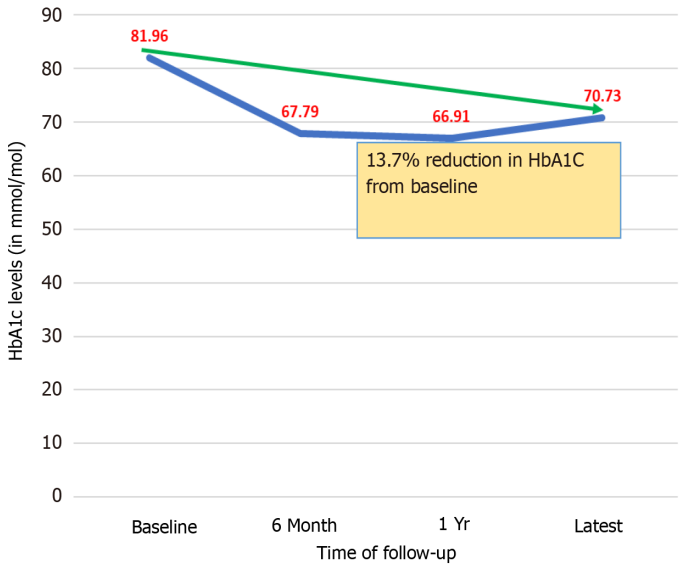

Figure 1 Illustrates the changes in glycated hemoglobin after starting semaglutide. The comparison between latest glycated hemoglobin and baseline showed a 13.7% reduction. However, in 1 year, it reached to 18.36% decline before it started rising again.

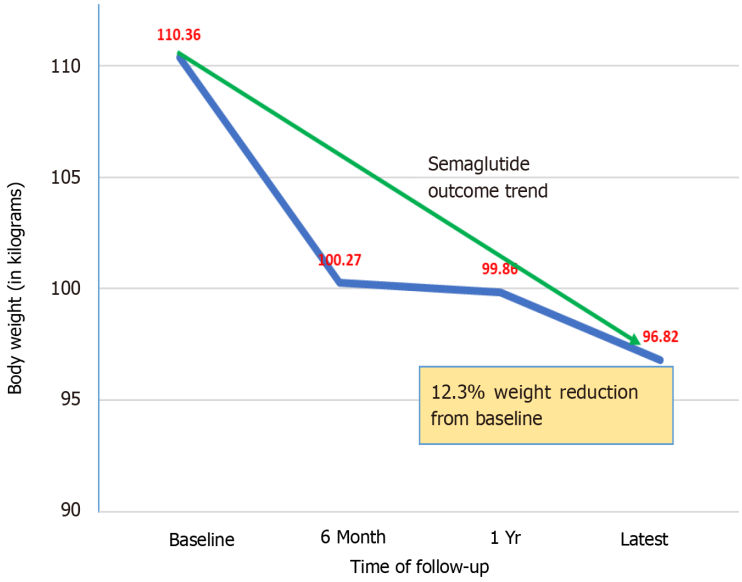

Figure 2 The weight reduction trend while using semaglutide for treatment of diabesity.

compared to placebo for treatment of patients with T2DM[5]. This study also very clearly showed better efficacy of this GLP-RA molecule compared to other antidiabetic medications for glycemic control with HbA1c reductions of 0.56% and 0.63% respectively when treated with 0.5 mg and 1.0 mg weekly of S/c semaglutide respectively. Our patients achieved greater mean HbA1c reduction (15.05 mmol/mol – equivalent to approximately 1.4%) at one year of follow up compared to the above meta-analysis-based study results for management of T2DM as all our patients were already on other antidiabetic medications. We acknowledge that our patients had higher baseline HbA1c (81.96 ± 20.96 mmol/mol) which could be the reason for better HbA1c reduction compared to the data revealed by clinical trials in which the baseline HbA1c was found to be comparatively lower (median HbA1c: 8.1–8.5%; 64.8–69.6 mmol/mol)[6]. Higher baseline HbA1c is known to be associated with better HbA1c reduction when treated with any antidiabetic agent including GLP-1RA as demonstrated by previous studies[7,8], which likely further explain our results with considerable HbA1c reduction. Moreover, other antidiabetic agents used by our patients (such as metformin and SGLT-2 inhibitors) would have resulted in better glycemic control. This is comparable to the observations from the SUSTAIN 9 trial, which showed that adding injectable semaglutide to patients treated for at least 90 days with SGLT-2i resulted in a significant reduction in HbA1c of an average of 1.42% (−15·55 mmol/mol)[9].

We observed a gradual worsening of metabolic control with an increase in HbA1c levels from 66.91 mmol/mol at one year to 70.73 mmol/mol in the last follow up at 2.6 ± 1.1 years. GLP-1RA medications were found to have lower efficacy in controlling chronic hyperglycemia in patients with T2DM after years of use because of various reasons such as

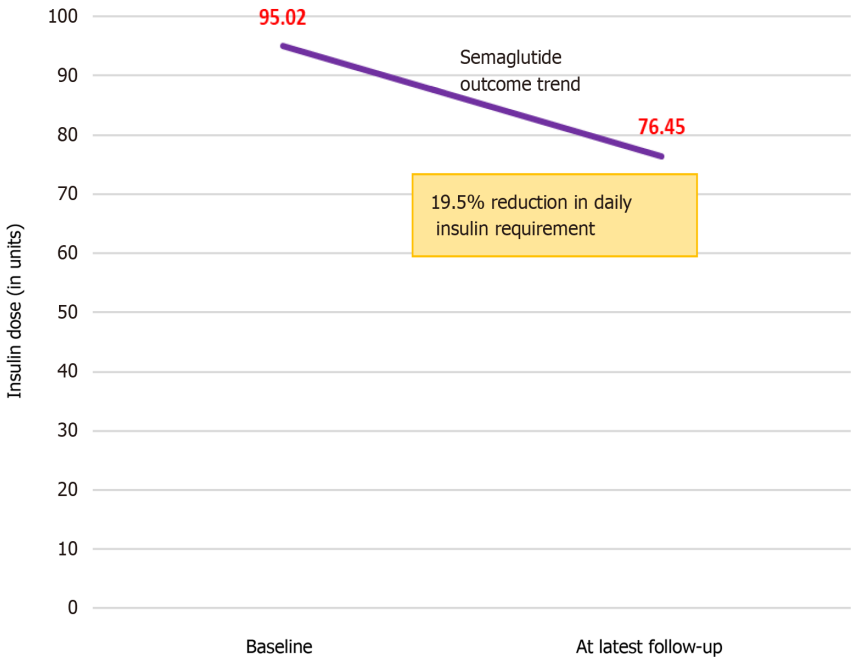

Figure 3 Displays the mean reduction in daily insulin requirement after initiating semaglutide.

antibody formation against some of the molecules, gradual decline in beta cell function is most patients with T2DM as part of the pathobiological behavior of the disease, and possibly a decrease in effector response to GLP-1RA drugs at the molecular level[10-13]. We believe that the slight worsening of metabolic control with a gradual increase in HbA1c level observed in our cohort at the latest follow-up could be explained by similar mechanisms.

Insulin dose reduction in a good proportion of patients was one of the interesting observations of our study which has great metabolic connotations in managing patients with diabesity. Marked improvement of glycemic control with semaglutide treatment helped insulin dose reduction in many patients and even total discontinuation of insulin in 4 patients in this cohort. Insulin dose reduction was reported by some of the RCTs and some cohort studies with the addition of semaglutide in the treatment regime[14-18]. However, we observed a mean insulin dose reduction from 95.02 to 76.45 units which is higher than the mean insulin dose reduction of 4-6 units observed by the SUSTAIN-5 Trial[15] and the study reported by Ares-Blanco *et al*[18] (16 units of insulin dose reduction) from Spain. Again high baseline HbA1c (82 mmol/mol) levels with a marked improvement in the metabolic control following initiation of semaglutide would explain this discrepancy, as other studies reported mean baseline HbA1c of 64 to 72 mmol/mol at the time of commencement of semaglutide treatment.

In our cohort we observed a weight reduction of 10.5 kg at 12 months' follow up with a further improvement of body weight by another -3.04 kg at the latest follow up (a total mean weight loss of 13.54 kg) at a mean 2.6 ± 1.1 years. The reported mean weight loss observed in major RCTs with semaglutide in patients with diabesity ranged from 2.32 to 3.99 kg in an updated meta-analysis[5]. Real-world data shows variable weight loss response ranging from 4.2 kg to 9.0 kg[19, 20]. Weight loss response in patients with T2DM following semaglutide therapy could be related to various factors such as baseline body weight, ethnic-specific incretin response and the glycemia-related fluctuations in incretin physiology [13]. Higher degree of weight loss observed in our cohort could also be due to the co-administered antidiabetic medications with synergistic weight loss potential (especially SGLT-2i) and significant insulin dose reductions.

The side effects experienced by patients in our study were mainly gastrointestinal and mild, which improved with ongoing treatment. The low rate of side effects we observed in this study compared to various other studies[5,21,22] could be related to the poor reporting of adverse events in the clinical records during follow up of the patients, a limitation unavoidable in retrospective studies.

Study limitations and implications for future research

We acknowledge a few limitations to our work which are inherent to retrospective cohort studies. Because of inadequate data, we had to exclude several cases from the analysis. Although several patients had at least annual follow-up prior to the last follow up we documented in our study, we were unable to get adequate data for analysis of these periodic follow-ups in obtaining meaningful statistical outcomes in this study. We were also unable to procure adequate data on cardiometabolic parameters such as improvement of blood pressure, lipid profile and renal outcomes because of inadequate documentation of these data in the follow-up period. Extrapolating the marked weight reduction of 12.3% into cardiometabolic outcomes from previous studies, we would have expected significant improvements in the above parameters which were not documented well in our patients' clinical records, and this remains as a major pitfall of the study. Inadequate reporting of adverse events in the case records could have been the reason for the low incidence of side

effects observed in this study.

However, the remarkable improvements in body weight, HbA1c and insulin dose reduction observed in our study bring forth the importance of appropriate use of GLP-1RA molecules including semaglutide in our day-to-day clinical practice. In fact, our data shows more profound improvements in diabesity outcomes such as marked weight loss and HbA1c reduction compared to RCT-based study data. This has important clinical implications for future research as significant weight loss in appropriately selected patients with early onset T2DM could result in diabetes remission or even reversal at least in a proportion of patients. Moreover, such patients may also have better cardiovascular benefits compared to patients with longer duration of diabetes. Therefore, compiling more real-world data with this kind of observational studies should enhance our current knowledge-base to inform better medical practice decision making in the future.

CONCLUSION

Our data shows that semaglutide use is associated with better clinical and biochemical outcomes in the real-world management of diabesity compared to the RCTs and other observational studies, with higher mean weight reduction of 12.3%, improvement of mean HbA1c by 14.5 mmol/mol (at one year), and mean insulin dose reduction of 18.6 units. Although we could not get adequate data on cardiometabolic outcomes, extrapolating the benefits of > 10% weight loss in clinical settings would have improved several of these outcomes. More data from real-world observational studies is expected to improve our understanding in using semaglutide and other newer GLP-1RA molecules for judicious management of diabesity to address the alarming rise in this clinical problem across the globe secondary to the obesity pandemic. Future research should evaluate the feasibility of early initiation of GLP-1RA such as semaglutide in patients with diabesity to see if that improves clinical and therapeutic outcomes.

ARTICLE HIGHLIGHTS

Research background

Diabesity, diabetes as a consequence of obesity, is a huge healthcare challenge across the globe and judicious use of antidiabetic medications like semaglutide is important for the optimal management as shown in randomized controlled trials (RCTs).

Research motivation

Real-world data on management of diabesity with semaglutide are also crucial for appropriate clinical practice decision making.

Research objectives

We aimed to study the real-world benefits and side effect profile of using semaglutide to manage patients with diabesity.

Research methods

In a retrospective study, we evaluated the efficacy and safety of semaglutide for managing patients with diabesity between January 2019 to May 2023 in a large academic hospital in the United Kingdom. With the relevant demographic information, we captured patients' data from the electronic case records on improvement of glycated hemoglobin (HbA1c), body weight reduction, and insulin dose adjustments at 6 and 12 months, as well as at the latest follow up period.

Research results

Among 106 patients (56 males) with T2DM with a mean age and diabetes duration 60.8 ± 11.2 years, 12.4 ± 7.2 years respectively treated with semaglutide for a mean 2.6 ± 1.1 years, significant improvements in diabesity outcomes such as a mean weight reduction of 12.3% and HbA1c reduction of 13.7% from baseline at the latest follow-up period were observed. A mean insulin dose reduction of 19.5% from baseline was also observed at the latest follow-up as an additional benefit of semaglutide treatment. Mild gastrointestinal side effects like bloating and nausea, improving with prolonged use of semaglutide were also observed in this study.

Research conclusions

As RCTs are performed in strictly controlled research environments, the results may not always reflect patient outcomes of real-world clinical practice settings. Reviews of large-scale cohort data from real-world settings as in our study would inform better clinical practice decision making to improve the care of patients with diabesity.

Research perspectives

Significant improvements in diabesity outcomes such as reductions in body weight, HbA1c, and insulin doses were observed with semaglutide treatment, without major adverse effects in a real-world clinical practice setting.

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FOOTNOTES

Author contributions: Alkhalifah M, Afsar H and Shyams A collected the clinical data. Alkhalifah M, Shyams A and Blaibel D performed literature search, and interpretation of relevant data following statistical analysis; Alkhalifah M, Blaibel D, and Chandrabalan V contributed to the work with additional literature review and revision of the article critically for important intellectual content; Chandrabalan V also procured the patient data from the hospital electronic records; Pappachan JM contributed to the conceptual design of the paper and critically supervised the whole drafting, literature review, revision and modifications of the paper including figure construction and is the final author; All authors have read and approved the final version of the manuscript.

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ORCID number: Joseph M Pappachan 0000-0003-0886-5255.

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ORIGINAL ARTICLE

Retrospective Study

Cluster sampling methodology to evaluate immunization coverage

Aishwarya Sharma, RB Jain, Jitesh Satija, Aditi Sharma, Avani Sharma, Sudhir Shekhawat

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Aishwarya Sharma, Department of Community Medicine, School of Medical Sciences & Research, Sharda University, Greater Noida 201306, UP, India

RB Jain, Department of Community Medicine, World College of Medical Sciences & Research, Jhajjar 124103, Haryana, India

Jitesh Satija, Department of Community Medicine, ESIC Medical College & Hospital, Faridabad 121012, Haryana, India

Aditi Sharma, Department of Community Medicine, Hamdard Institute of Medical Sciences & Research, New Delhi 110062, India

Avani Sharma, Department of Community Medicine, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak 124001, Haryana, India

Sudhir Shekhawat, Independent Biostatistician, Ghaziabad 201014, UP, India

Corresponding author: Aishwarya Sharma, MD, Senior Resident, Department of Community Medicine, School of Medical Sciences & Research, Sharda University, Knowledge Park 3, Greater Noida 201306, UP, India. aishwarya_712@yahoo.in

Abstract

BACKGROUND

Immunization is a key component of primary health care and an indisputable human right. Vaccines are critical to the prevention and control of infectious disease outbreaks. The coronavirus disease 2019 (COVID-19) pandemic and associated disruptions over the past two years have strained the health systems, with many children missing out on essential childhood vaccines.

AIM

To evaluate the immunization coverage among 12-23-month-old children in the rural areas of Community Health Centre (CHC) Dighal and to determine the factors influencing the existing immunization coverage.

METHODS

A coverage evaluation survey was conducted according to the 30-cluster sampling technique, which is the standard methodology for such surveys devised by World Health Organization. A total of 300 children aged 12-23 months were included, whose immunization details were noted from their immunization cards.

RESULTS
Full immunization rate was noted in 86.7% of the children, with partial and non-immunized children accounting for 9% and 4.3% respectively. The full immunization dropout rate was 4.2%. The common reasons for partial or non-immunization were family problem including illness of mother, vaccine not being available and child being ill. Place of birth (P = 0.014) and availability of immunization card (P < 0.001) were significant predictors of the immunization status. Since the study was conducted in 2020/2021, health services were disrupted due to the COVID-19 lockdown.

CONCLUSION
Due to the coverage being higher than the national average, it was concluded that the immunization coverage was optimal and not affected by the COVID-19 pandemic.

Key Words: Immunization coverage; Children; COVID 19 pandemic; Vaccines; Family health; Routine immunization

Core Tip: Cluster sampling technique is a unique method of probability sampling. It has immense scope in being utilized for healthcare delivery service coverage. Each cluster is crucial in representing a geographically diverse population under study. This sustains uniform representation along with statistical correctness. This technique has been employed to evaluate the immunization coverage among children in a rural setting in India, during the pandemic.

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INTRODUCTION

Immunization is a key component of primary health care and an indisputable human right. Immunization currently prevents 3.5-5 million deaths annually from diseases like diphtheria, tetanus, pertussis, influenza and measles[1]. Despite tremendous progress, vaccination coverage has plateaued in recent years and even dropped for the first time in a decade in 2020, with 23 million children missing their vaccination due to the coronavirus disease 2019 (COVID-19) pandemic[2]. Global immunization efforts have saved at least 154 million lives or an equivalent of 6 lives every minute every year, predominantly infants. Measles vaccination has been the most impactful in reducing infant mortality, accounting for 60% of the lives saved due to immunization[3].

Under the Universal Immunization Programme, the Government of India provides vaccination to prevent seven vaccine-preventable diseases, *viz*., diphtheria, pertussis, tetanus, polio, measles, a severe form of childhood tuberculosis and hepatitis B, haemophilus influenza type b and diarrhoea[4]. The immunization coverage for 12-23-month-old children under the National Family Health Survey (NFHS 5) is 83.8 %, reflecting a 5.9% increase from NFHS 4 figure[5]. In rural Haryana, the coverage is 80.8%[6], with the Jhajjar district recording a massive increase in coverage of 24.5% to 84.1%[7].

Immunization against vaccine-preventable diseases is a cost-effective and efficient tool to reduce morbidity and mortality in children. Assessing the immunization coverage offers an idea about the extent of its utilization, the beneficial impact of the vaccination program and planning appropriate action to enhance its overall efficiency. This has enhanced healthy survival in children. During the COVID-19 pandemic, routine immunization services were disrupted due to the lockdown. Though many studies were conducted on immunization coverage, few were carried out in the pandemic setting, especially in rural India. This study throws light on the current situation of immunization coverage in the study area, despite the constraints posed by the pandemic. Hence, the current study was planned to evaluate the immunization coverage in the rural areas of Community Health Centre (CHC) Dighal, district Jhajjar, Haryana, which is the field practice area attached to the Department of Community Medicine, Post-graduate Institute of Medical Sciences Rohtak. The study aimed to evaluate the immunization coverage among 12-23-month-old children in the rural areas of CHC Dighal and to determine the factors influencing it.

MATERIALS AND METHODS

Study area

The study was conducted in the rural area of the CHC, Dighal (District Jhajjar), which is the field practice area attached to the Department of Community Medicine, Pt. B.D. Sharma Post Graduate Institute of Medical Sciences, Rohtak. Twenty-

six villages were provided health services through a network of five primary health centres – Dighal, Dujana, Kharhar, Barhana, and Bhambewa (Figure 1). The population of this block was 106654 as per the record of the CHC area till April 2020.

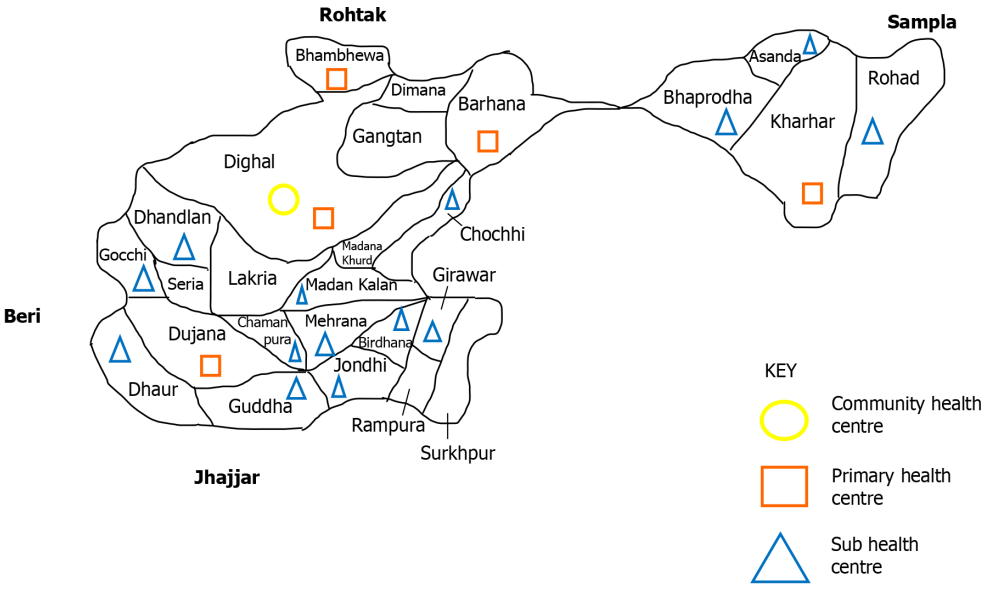

Figure 1 Map of Community Health Centre Dighal, Block Beri.

Study design

The present study was a cross-sectional, community-based study conducted from August 2021 to July 2022.

Inclusion criteria

All children aged 12-23 months residing in the study area and children whose parents were willing to give written informed consent to participate in the study were included.

Exclusion criteria

Severely sick children and parents of the children under the study who were unable to give relevant information for the study were excluded.

Data collection

The coverage evaluation survey in the area was conducted according to the 30-cluster sampling technique, the standard methodology for such surveys devised by World Health Organization[8]. Ten children aged 12-23 months were selected from each of the selected clusters. If there were more than one eligible subject in any household, all the subjects were enrolled in the study. This sampling design estimated immunization coverage to within + 10 percentage points of true proportion, with 95% confidence. The 30 by 10 cluster survey was a two-stage cluster sampling. In the first stage, all the villages in the area were listed alphabetically. The population of each village was listed alphabetically and cumulative populations were calculated. The sampling interval was calculated by using the formula: Total cumulative population/30 (cluster) = sampling interval.

A four-digit random number was selected from the digits of any currency note, which was equal to or less than the sampling interval. Cluster no 1 was identified by locating the first village whose population was equal to or more than the random number selected. Cluster number 2 was identified by using the formula: Random number + sampling interval = -.

The cumulative population listed for that village was equal to or exceeded the calculated number. Clusters number 3, 4, 5 and so onwards were identified and located by using the formula: Number which identified the + sampling interval = -, location of the previous cluster.

In the second stage of the cluster survey, the investigator chose a central point in the village and conducted the survey thereafter from house to house, till the desired sample size was reached, moving on to the adjacent street, if necessary (Figure 2A). In the next cluster, the investigator started from the periphery of the village, just to have a uniform sample of the total population (Figure 2B). This approach was adopted unlike the procedure for the previous cluster so that no particular section of the population was included in the study.

The investigator herself conducted the study through house-to-house visits and all the parents of the study subjects were fully informed about the purpose of the study. Written informed consent was obtained from the individuals before conducting the interview. Spot maps for two villages covered: Madana Kalan and Dhandlan (Figure 3).

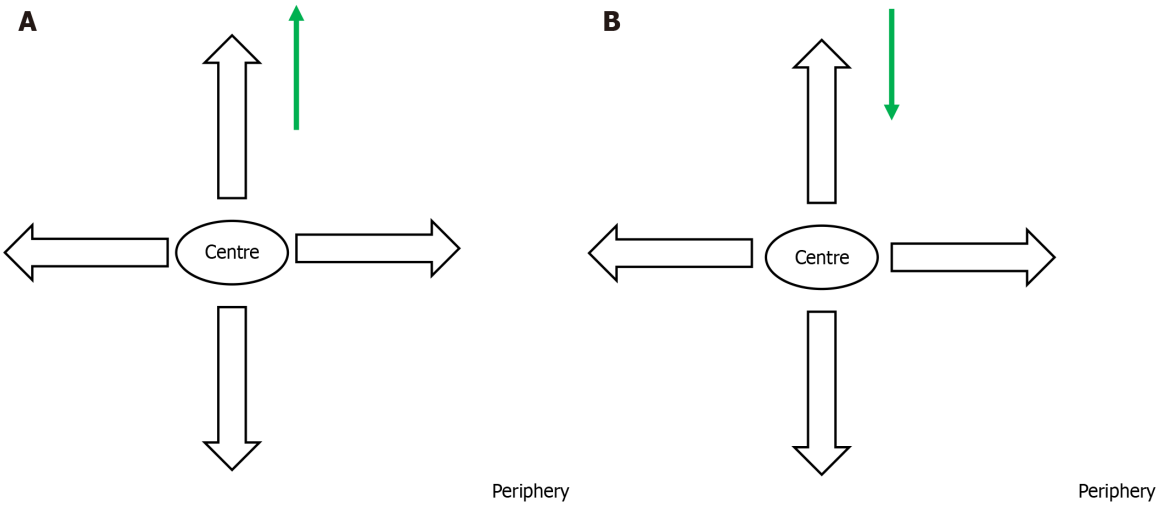

Figure 2 From centre towards periphery of the village and from periphery to centre of the village. A: From centre towards periphery of the village; B: From periphery to centre of the village.

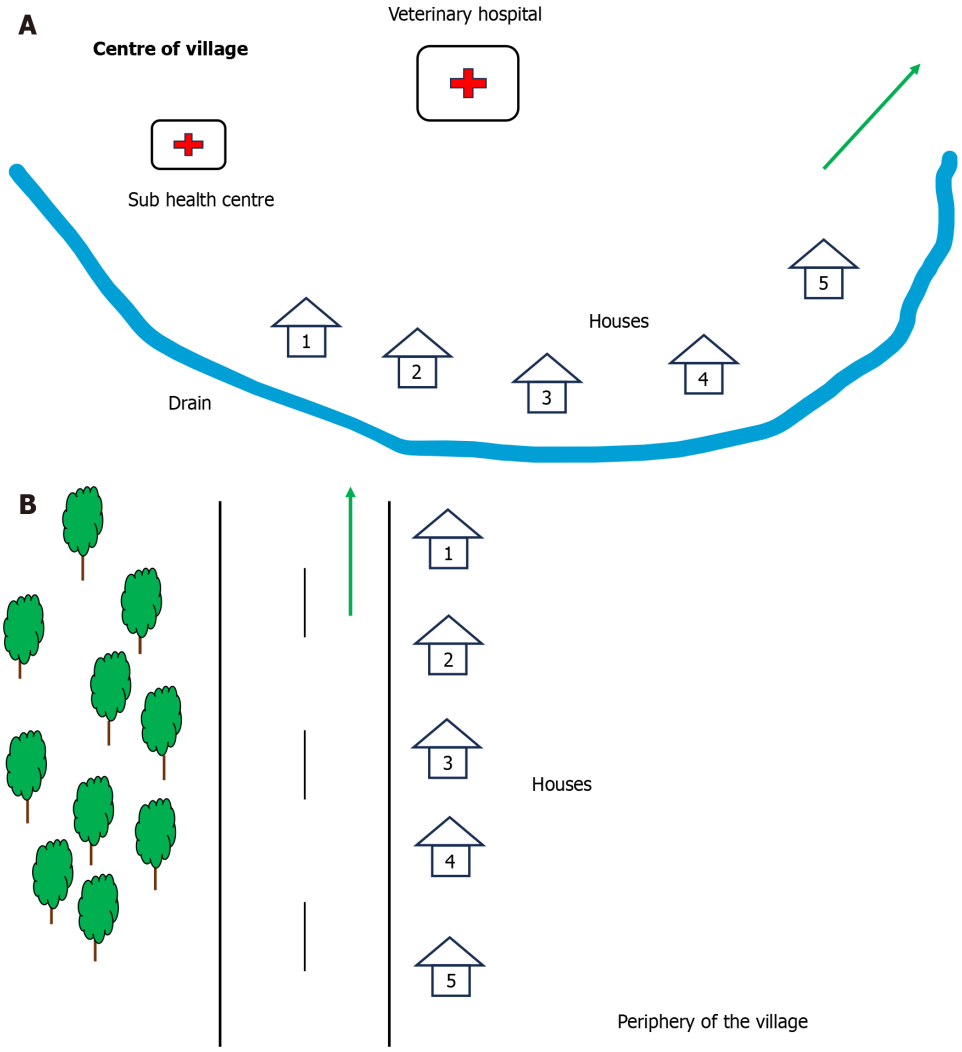

Figure 3 Spot map for Madana Kalan village and Dhandlan village. A: Madana Kalan village; B: Dhandlan village.

Data collection tool

This was an interview-based study and a semi-structured interview schedule based on the World Health Organization Universal Immunization Programme Coverage Household Survey form 2018, as relevant to the present study and incorporating questions related to the COVID-19 pandemic[8]. Vaccination cards were used to know the exact time of vaccination and in case cards were not available, history from either parent (mother/father)/reliable respondent was obtained and matched with the immunization record of the respective sub-centre. The reasons for refusal and dropouts were noted.

Operational definitions

A Fully Immunized child is one who had received one dose of Bacillus Calmette Guerin (BCG), three doses of diphtheria pertussis tetanus (DPT) and oral polio virus (OPV) and one dose of Measles vaccine before one year of age. A partially immunized child is one who had been administered a vaccine but whose immunization is not complete. A non-immunized child is one who had not been given even a single dose of vaccine.

Dropout rates were calculated using the following formulae

For full immunization dropouts: [(BCG - Measles) × 100]/BCG. For pentavalent (PENTA)/OPV/rotavirus (ROTA) virus vaccine dropouts: [(PENTA1 - PENTA3) × 100]/PENTA1.

Statistical analysis

The data so collected was compiled and entered as a master chart in an MS Excel spreadsheet. Analysis was carried out using the Statistical Package for the Social Science (SPSS) Version 20 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp). Data were analyzed using descriptive statistics, χ^2 test and logistic regression statistical tests.

RESULTS

The total population covered (Table 1) comprised 53.7% male and 46.3% female children). The government hospital was where the majority of births took place. The median birth weight was 2.75 (1.6-4.0) kg. Most of the children were of the first or second birth order (86%). The mothers were mostly educated till senior secondary school (30.3%) or graduates (25.7%) and were mostly housewives (65.7%). Similarly, fathers were educated up to senior secondary school (32%) or graduation (34%) and commonly worked in the private sector (23.3%), followed by the labor market (22.7%). The joint family system was common in the area (59.7%). The majority of the children belonged to lower middle class and lower-class socioeconomic conditions, according to the Modified BG Prasad Socioeconomic Scale (Version 2020)[9] (Table 1). Immunization card was available with 94% of the parents. BCG scar was noted in 82% of the children. Most children took their vaccination at a government setup (97.6%). The full immunization rate was 86.7% with only 9% and 4.3% of the children being partially or non-immunized respectively.

The COVID-19 pandemic started in March 2020 and India is still witnessing a few hundred cases and deaths even today. The months of March to May 2020 witnessed a nationwide lockdown. A large number of migrant laborers traversed large stretches of the country to return to their native towns and villages[10]. From June 2020 onwards, there was relaxation in a few parts of the country, where transmission was minimal. The process of unlocking went on till December 2020. With the second wave hitting the country in March 2021, a nationwide lockdown was imposed again from April to June 2021, extending in some states, including Haryana till August 2021[11].

Vaccination services were hit, apart from other essential childhood care services like the Anganwadi centers. There was no vaccination at certain times due to the vaccination center being located in a hotspot, being declared a containment zone. Numerous people tested positive for the virus and were unable to get their children vaccinated during the periods of illness when they were quarantined in their homes. The mass movement of migrant laborers returning to their villages resulted in an increase in cases in rural areas. The contacts were advised to quarantine for 14 d, later reduced to 7 d. Those with a travel history were also quarantined. This resulted in their children missing essential childhood vaccination. Rumors were rife during the pandemic. This was a very important reason for children missing timely vaccination.

The immunization coverage from the present study suggests that the COVID-19 pandemic did not significantly affect the vaccination drive. The parents took their responsibility of getting their children immunized seriously. This further revealed the efforts undertaken by the peripheral health workers, who compensated for any hurdles faced due to the lockdown, managing timely immunization of the children.

Reasons for missing vaccination

A total of 210 children out of 300 missed one or the other vaccine mentioned in the National Immunization Schedule. The reasons for missing out any vaccine (n = 210), were lack of information (7.6%), lack of motivation (8.1%), obstacles like unavailability of the vaccine or the vaccinator (69.1%) and factors related to the COVID-19 pandemic (15.2%). The coverage for different vaccines is shown in Table 2. BCG, OPV, PENTA and ROTA had coverage > 90%. Measles rubella (MR) 1 had good coverage of 91.3%. MR 2 could be given between 16-24 months. Since the median age of the study participants was 19 months, some children still had to take the second dose of MR vaccine. Hence, its coverage could increase in the upcoming months. Hepatitis B and OPV birth doses had poor coverage, at 59% and 69.7% respectively. As the booster doses (DPT-B and OPV-B) could be given up to 2 years of age, the children still had time to be administered

Table 1 Socio-demographic profile of study subjects

Sociodemographic variable	Number	Percentage (%)
Gender		
Male	161	53.7
Female	139	46.3
Place of birth		
Government hospital	195	65.0
Private hospital	99	33.0
Home	6	2.0
Age (months)		
12-17	108	36
18-23	192	64
Birth weight		
< 2.5 kg	99	33
≥ 2.5 kg	201	67
Birth order		
≤ 2	252	86.0
> 2	48	14.0
Mother's literacy level		
Illiterate	2	0.7
Primary school (0-5 std)	9	3.0
Middle school (6-8 std)	30	10.0
High school (9-10 std)	67	22.3
Senior secondary school (11-12 std)	91	30.3
Graduate	77	25.7
Post-graduate	24	8.0
Mother's occupation		
Self-employed	10	3.3
Government job	11	3.7
Private job	10	3.3
Farmer	37	12.3
Laborer	35	11.7
Housewife	197	65.7
Father's literacy level		
Illiterate	1	0.3
Primary school (0-5 std)	3	1.0
Middle school (6-8 std)	40	13.3
High school (9-10 std)	56	18.7
Senior secondary school (11-12 std)	96	32.0
Graduate	102	34.0
Post-graduate	2	0.7
Father's occupation		
Unemployed	11	3.7

Self-employed	57	19.0
Government job	60	20.0
Private job	70	23.3
Farmer	34	11.3
Laborer	68	22.7
Type of family		
Nuclear	37	12.3
Joint	179	59.7
Three-generation	84	28.0
Socio-economic class		
Upper class	15	5.0
Upper middle class	59	19.7
Middle class	54	18.0
Lower middle class	90	30.0
Lower class	82	27.3

Table 2 Coverage of different vaccines among the study subjects

Vaccine		Number	Percentage (%)
BCG		286	95.3
OPV	OPV-1	282	94.0
	OPV-2	281	93.7
	OPV-3	280	93.3
PENTA	PENTA-1	283	94.3
	PENTA-2	282	94.0
	PENTA-3	282	94.0
ROTA	ROTA1	282	94.0
	ROTA2	280	93.3
	ROTA3	277	92.3
f IPV	f IPV-1	280	93.3
	f IPV-2	274	82.3
PCV-1	PCV-1	270	90.0
	PCV-2	255	85.0
MR	MR1	274	91.3
	MR2	152	50.7
HEP B-0		177	59
OPV-0		209	69.7
PCV-B		239	79.7
DPT-B		151	50.3
OPV-B		151	50.3

BCG: Bacillus Calmette Guerin; OPV: Oral polio virus; PENTA: Pentavalent; ROTA: Rotavirus; f IPV: Fractional inactivated polio virus; PCV: Pneumococcal conjugate vaccine; MR: Measles rubella; HEP B: Hepatitis B; B: Booster dose; DPT: Diphtheria pertussis tetanus.

the same (Table 2). The dropout rates for different vaccines have been calculated as 1.05%, 0.35%, 0.71%, 1.78%, 3.18% for BCG-PENTA1, PENTA1-PENTA3, OPV1-OPV3, ROTA1-ROTA3, PENTA1-Measles dropouts. Full immunization dropout (BCG-Measles) was 4.2%.

The association of variables with immunization coverage is shown in Table 3. Age (months), socioeconomic status, type of family and place of birth had a significant association ($P < 0.05$) with immunization coverage. The presence of BCG scar and the availability of immunization cards were highly significant indicators of immunization coverage ($P < 0.001$) (Table 3). However, in logistic regression analysis (Table 4), place of birth ($P = 0.014$) and availability of immunization card ($P < 0.001$) were found significant predictors of immunization status (Table 4).

DISCUSSION

The present study reports the full immunization coverage as 86.7%, which is higher than the national average of 83.8% [5], revealing that priority was given to the Routine Immunization Programme, with regular sessions at a fixed date, time and venues. People of the area were aware of and accepted the Immunization Programme. The factors significantly associated with the coverage were the place of birth and the presence of a BCG scar.

The present study concluded that the majority of the participants were born in a government hospital (66.3%). There were no home deliveries. In a similar study conducted by Devasenapathy *et al*[12] in Delhi, only 73% of the children were born in a facility (government or private), as the study was conducted in urban slums. The association between place of birth and immunization status was found to be significant in the current research ($P = 0.022$) ($P < 0.01$).

In the present study, the median age of the study subjects was 19 months. In a study by Adedire *et al*[13] from Nigeria, 23.5%, 30.1%, and 46.4% of the children were in the age group of 12-15, 16-19 and 20-23 months respectively. Muluye *et al* [14] conducted a study in Ethiopia revealing 34.3%, 29.4%, 24.3%, and 12% of children in the 12-14, 15-17, 18-20- and 21-23-months age groups. A study from Indonesia by Herliana and Douiri[15] reported 28.8%, 24.6%, 23.3%, and 23.3% of children in the 12-23, 24-35, 36-47 and 48-59-months age groups. The association between the current age of the study subjects and immunization status proved to be significant in the current research ($P = 0.048$). Similarly, Herliana and Douiri[15] reported a significant association.

The joint family was the main type of family in the present study, at 59.7%. With high immunization coverage, this could suggest grandparents and relatives spending time on the child's health care, in case the mother was unable to do so. In contrast, Devasenapathy *et al*[12] found the nuclear type to be more common (71.5%). This could be due to the study being conducted in urban slums. The current research found a significant association for the above ($P = 0.039$). Conversely, the above association was not significant in the study of Kumar *et al*[16].

Most of the study subjects belong to the lower middle- and middle class in this study. Still, with good immunization coverage (86.7%), it can be said that the health services were functional, accessible and acceptable to all strata of society. Similarly, class IV was the predominant socio-economic class in a study from Tripura by Datta *et al*[17]. The present study found the above association significant ($P = 0.026$). Kulkarni and Chavan[18] too found the association highly significant ($P < 0.001$).

About 94% of the study participants had the immunization card with them. This showed that parents understood the importance of maintaining vital health care documents. It was a valid piece of evidence marking the antigens already vaccinated against. This eliminated any duplications or omissions in the immunization of children. Similar findings were reported by Gupta *et al*[19]. About 84% of the parents had immunization cards with them. The present study identified a highly significant association between the availability of immunization cards and immunization status ($P < 0.001$). It was a valid piece of evidence marking the antigens already vaccinated against. Chhabra *et al*[20] too found the association significant ($P < 0.01$).

The BCG vaccine left a scar over the vaccination site (the deltoid muscle). This was an immunological response to the antigen. The BCG scar serves as a surrogate marker for vaccination against tuberculin antigen. It may be normally absent in up to 20% of the children[21]. Its absence does not necessarily signify immunization failure against TB antigen. This study finds 82% of children having the BCG scar. The present study found a highly significant association between the availability of immunization cards and immunization status ($P < 0.001$). Gupta *et al*[19] too found the association highly significant ($P = 0.000$).

Upon applying logistic regression analysis in the present study, the place of birth ($P = 0.014$) and availability of immunization cards ($P < 0.001$) were found to be significant factors for full immunization status. These factors revealed adequate access and acceptance of health services being provided in the study area. Saikia *et al*[22] highlighted that possession of child's health card is the most significant factor for reducing the disparities in immunization coverage in India. In a study from Nigeria, Adedire *et al*[13] reported attendance of mothers at antenatal care centers [adjusted odds ratio (aOR) = 3.3, 95% confidence interval (CI): 1.1-8.3], maternal tetanus toxoid immunization (aOR = 3.2, 95%CI: 1.1-10.0), access to immunization information (aOR = 1.8, 95%CI: 1.1-2.5) and good knowledge of immunization in mothers (aOR = 2.4, 95%CI: 1.6-3.8) as significant determinants of full immunization.

Periodic intensification of routine immunization *via* Intensified Mission Indradhanush has improved vaccination coverage and timeliness[23]. Mobile messaging services such as the Kilkari application are an important source of awareness, timeliness and uptake of immunization services[24]. Newer tools such as automated incentivised mobile phone reminders, immunization due-list, computerized data tracking, community mobilization and campaigns improved vaccine coverage. Future work is needed to evaluate the effectiveness of identified technologies across diverse settings in India[25].

Table 3 Association of variables with the immunization status of the study subjects

Characteristics	Partial & non immunized (*n* = 40)	Immunized (*n* = 260)	OR (95%CI)	*P* value
Gender				
Male	24	137	1.35 (0.68-2.65)	0.388
Female	16	123	1 (ref)	
Age (months)				
12-17	20	88	1.96 (1.00-3.82)	0.048[a]
18-23	20	172	1 (ref)	
Birth weight (kg)				
< 2.5	14	85	1.11 (0.55-2.23)	0.773
≥ 2.5	26	175	1 (ref)	
Mother's education				
Illiterate/up to 8th std	6	35	1.13 (0.44-2.90)	0.792
9th std. & above	34	225	1 (ref)	
Mother's profession				
Employed (government/private job)	3	18	1.09 (0.31-3.88)	0.894
Others[1]	37	242	1 (ref)	
Father's education				
Illiterate/up to 8th std	6	38	1.03 (0.41-2.70)	0.553
9th std & above	34	222	1 (ref)	
Father's occupation				
Employed (government/private job)	16	114	0.85 (0.43-1.68)	0.648
Others[1]	24	146	1 (ref)	
Socio-economic status				
Lower/lower middle class	29	143	2.16 (1.03-4.50)	0.026[a]
Middle/upper middle/high class				
Type of family				
Joint/three generations	31	232	0.42 (0.18-096)	0.039[a]
Nuclear	9	28	1 (ref)	
Birth order				
> 2	9	39	1.64 (0.73-3.72)	0.228
≤ 2	31	221	1 (ref)	
Availability of immunization card				
No	13	5	3.40 (1.65-7.02)	< 0.001[b]
Yes	27	255	1 (ref)	
Place of birth				
Home/private hospital	8	97	0.42 (0.19-0.95)	0.022[a]
Government hospital	32	163	1 (ref)	
BCG scar				
No	15	39	3.40 (1.65-7.02)	0.001[b]
Yes	25	221	1 (ref)	

[a]$P < 0.05$.

[b]*P* < 0.001.
[1]Self-employed/housewife/farmer/laborer.
BCG: Bacillus Calmette Guerin; OR: Odds ratio; CI: Confidence interval.

Table 4 Logistic regression model for a characteristic associated with Immunization status

Characteristics	Adjusted OR (95%CI)	*P* value
Place of birth	0.386 (0.181-0.824)	0.014[a]
Availability of immunization card	18.807 (9.104-38.854)	< 0.001[b]

[a]*P* < 0.05.
[b]*P* < 0.001.
OR: Odds ratio; CI: Confidence interval.

Recommendations
An area of particular interest for research can be previous adverse events causing fear in the community about vaccination. Parents whose children have been vaccinated and are doing well can be motivated to come forward and engage with parents having any apprehensions. The panchayat or village body has a great deal of support and is looked up to in the area. If its members pledge their support to routine immunization, many hurdles can be overcome.

Limitations
The study was conducted in a rural block attached to a tertiary care hospital, and teaching and training center. Hence, the results cannot be generalized. Owing to the cross-sectional nature of the study, the direction of association can't be ascertained.

CONCLUSION
The immunization coverage in the study area was significantly high and better than the national average, with minimal dropout rates. Priority was given to the Routine Immunization Program. The COVID-19 pandemic did not have any significant impact on immunization coverage. The parents were responsible for keeping abreast with the immunization schedule of their children. The health workers worked diligently to compensate for the missed vaccination during the lockdown period.

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FOOTNOTES
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Institutional review board statement: The study was approved by the Biomedical Research Ethics Committee Pt. B.D. Sharma Post Graduate Institute of Medical Sciences/University of Health Sciences Rohtak with approval number BREC/Th/20/Comm. Med./02.

Informed consent statement: All study participants provided written informed consent prior to study enrollment.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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Country of origin: India

ORCID number: Aishwarya Sharma 0000-0002-5338-2650; Jitesh Satija 0000-0002-3699-5113; Aditi Sharma 0009-0006-2034-5163; Avani Sharma 0009-0001-5687-3674.

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ORIGINAL ARTICLE

Observational Study

Ensemble for evaluating diagnostic efficacy of non-invasive indices in predicting liver fibrosis in untreated hepatitis C virus population

Navneet Kaur, Gitanjali Goyal, Ravinder Garg, Chaitanya Tapasvi, Umit Demirbaga

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Navneet Kaur, Department of Biochemistry, Guru Gobind Singh Medical College and Hospital, Faridkot 151203, Punjab, India

Gitanjali Goyal, Department of Biochemistry, All India Institute of Medical Sciences, Bathinda 151005, Punjab, India

Ravinder Garg, Department of Medicine, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Faridkot 151203, Punjab, India

Chaitanya Tapasvi, Department of Radiodiagnosis, Guru Gobind Singh Medical College and Hospital, Baba Farid University of Health Sciences, Faridkot 151203, India

Umit Demirbaga, Department of Computer Engineering, Bartin University, Bartin 74100, Türkiye

Umit Demirbaga, Department of Medicine, University of Cambridge, Cambridge CB2 0QQ, United Kingdom

Umit Demirbaga, European Bioinformatics Institute, Wellcome Genome, Cambridge CB10 1SD, United Kingdom

Corresponding author: Navneet Kaur, MSc, Research Fellow, Department of Biochemistry, Guru Gobind Singh Medical College and Hospital, Sadiq Road, Faridkot- 151203, Faridkot 151203, Punjab, India. navmann23@yahoo.com

Abstract

BACKGROUND

Hepatitis C virus (HCV) infection progresses through various phases, starting with inflammation and ending with hepatocellular carcinoma. There are several invasive and non-invasive methods to diagnose chronic HCV infection. The invasive methods have their benefits but are linked to morbidity and complications. Thus, it is important to analyze the potential of non-invasive methods as an alternative. Shear wave elastography (SWE) is a non-invasive imaging tool widely validated in clinical and research studies as a surrogate marker of liver fibrosis. Liver fibrosis determination by invasive liver biopsy and non-invasive SWE agree closely in clinical studies and therefore both are gold standards.

AIM

To analyzed the diagnostic efficacy of non-invasive indices [serum fibronectin,

aspartate aminotransferase to platelet ratio index (APRI), alanine aminotransferase ratio (AAR), and fibrosis-4 (FIB-4)] in relation to SWE. We have used an Artificial Intelligence method to predict the severity of liver fibrosis and uncover the complex relationship between non-invasive indices and fibrosis severity.

METHODS

We have conducted a hospital-based study considering 100 untreated patients detected as HCV positive using a quantitative Real-Time Polymerase Chain Reaction assay. We performed statistical and probabilistic analyses to determine the relationship between non-invasive indices and the severity of fibrosis. We also used standard diagnostic methods to measure the diagnostic accuracy for all the subjects.

RESULTS

The results of our study showed that fibronectin is a highly accurate diagnostic tool for predicting fibrosis stages (mild, moderate, and severe). This was based on its sensitivity (100%, 92.2%, 96.2%), specificity (96%, 100%, 98.6%), Youden's index (0.960, 0.922, 0.948), area under receiver operating characteristic curve (0.999, 0.993, 0.922), and Likelihood test (LR+ > 10 and LR- < 0.1). Additionally, our Bayesian Network analysis revealed that fibronectin (> 200), AAR (> 1), APRI (> 3), and FIB-4 (> 4) were all strongly associated with patients who had severe fibrosis, with a 100% probability.

CONCLUSION

We have found a strong correlation between fibronectin and liver fibrosis progression in HCV patients. Additionally, we observed that the severity of liver fibrosis increases with an increase in the non-invasive indices that we investigated.

Key Words: Hepatitis C virus; Non-invasive biomarkers; Shear wave elastography; Fibronectin; Bayesian network; Machine learning; Liver fibrosis

Core Tip: The role of non-invasive indices (including serum fibronectin) was investigated to assess and differentiate liver fibrosis in untreated hepatitis C virus (HCV)-infected patients. The overall assessment and prediction process involved the correlation of fibronectin, alanine aminotransferase ratio, aspartate aminotransferase to platelet ratio index, and fibrosis-4 with severity staging performed through shear wave elastography. The role of non-invasive indices to assess and differentiate liver fibrosis is further validated through the calculation of diagnostic accuracy measured using various standard methods such as, sensitivity and specificity, Youden's index, area under receiver operating characteristic curve, and likelihood test. We have explored machine learning-based analysis using a Bayesian Network to predict and validate the diagnostic ability of non-invasive indices for predicting liver fibrosis in HCV patients.

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INTRODUCTION

Hepatitis C virus (HCV) is linked to both acute and chronic hepatitis, which can be of varying severity ranging from a mild infection to a severe and long-lasting infection with the possibility of cirrhosis and cancer. According to the fact sheet published by World Health Organization (WHO), approximately 58 million people are living with chronic HCV infection in 2019; this includes approximately 1.5 million new infections every year[1,2]. In this fact sheet, it was approximated by WHO that HCV infection resulted in around 290000 deaths in 2019, mostly linked to cirrhosis and hepatocellular carcinoma. HCV is a blood-borne, enveloped, and single-strand ribonucleic acid (RNA) virus with at least six genotypes and numerous subtypes; it belongs to the hepacivirus genus in the Flaviviridae family[3,4]. The prevalence of HCV genotypes and subtypes varies geographically according to transmission and ethnicity. A significant portion of the Indian population is infected with HCV, with a prevalence ranging between 0.5% and 1.5%. In India, some areas (Punjab and the north-eastern region) may represent HCV hotspots compared to other parts of India. Here, the most common means of HCV transmission are related to blood transfusion and the insecure use of injections for therapeutic reasons[5].

HCV does not cause any significant symptoms, and people often remain unaware of the advancement of the infection. The liver disease arising from the HCV infection gradually progresses through various phases, including inflammation, fibrosis, cirrhosis, and hepatocellular carcinoma. In the first phase, the liver becomes tender and expanded, depicting the immune system's response to the offending toxins. The second phase (fibrosis) is triggered through chronic (long-term) inflammation, usually as a result of the healing process of the liver to regenerate the damaged areas of the liver. At a

certain point, the liver reaches the stage of scarring and goes beyond its self-healing ability. This phase is known as cirrhosis. Interventions at the early stage of cirrhosis can stimulate healing and recovery from the infection. However, at a later stage and even when it reaches the final phase (carcinoma), the cells can not heal, which may lead to complete liver failure and eventually death. Looking into the progression phase, it is evident that by tracing the infection at the fibrosis phase, the possibility of improving the self-healing ability of the liver and stimulating the recovery process can be largely possible[6].

Thus, to diagnose chronic HCV infection at an early stage, invasive methods and non-invasive indices have been proposed and employed in clinical studies. *Liver biopsy* is the primary invasive method (often called a gold standard) to detect liver fibrosis. Although it has benefits, it is also linked to morbidity and complications (minor or significant); approximately a quarter of patients undergoing liver biopsy witness right upper quadrant pain[7]. In contrast, the non-invasive (blood-based) biomarkers proffer higher degree of patient acceptance over liver biopsy, alongside being safe and cost-effective. They are classified into two categories, serum biomarkers and imaging techniques.

Serum biomarkers are classified into two further forms, *i.e.* direct (class I) and indirect (class II) markers/indices. The class I indices are the direct fragments of components in the liver matrix. These fragments are produced by hepatic stellate cells during the remodeling process of the extra cellular matrix (ECM), hence reflecting the discharge of ECM. The class I indices (direct markers) are fibronectin, YKL-40, hyaluronic acid, laminin, procollagen type I carboxy-terminal peptide, and alpha-2-macroglobulin[8]. Among these, fibronectin is a glycoprotein of the ECM with a high molecular weight. Hepatocytes are the main cells responsible for a variety of cellular functions and protein synthesis. Serum fibronectin exists in two forms in the blood, *i.e.* cellular fibronectin and plasma fibronectin[9]. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and serum bilirubin are the mainly considered class II (indirect) indices in HCV, but they cannot distinguish between intermediate fibrosis stages[10].

Imaging techniques such as Fibroscan or transient elastography and shear wave elastography (SWE) are non-invasive methods to assess liver fibrosis. Although Fibroscan is prevalent in the United States, it has several limitations including cost of the equipment and lack of standardized cutoffs for the diagnosis of fibrosis stages. SWE is a non-invasive imaging tool that measures liver stiffness that, in turn, has been validated in clinical and research studies[11,12] as a surrogate marker of liver fibrosis. This technique can help to gather real-time images through a B-mode ultrasound probe[13]. Liver fibrosis determination by invasive biopsy and non-invasive SWE agree closely; thus, both methods are considered to be gold standards[14-16]. Existing studies[17-19] strongly suggest that SWE is accurate and has diagnostic effectiveness in predicting and staging biopsy-proven liver fibrosis patients across varied populations worldwide.

The interest in non-invasive markers or indices for predicting fibrosis in chronic HCV subjects has recently increased. However, these indices' validity is limited, restricting their adoption in clinical applications. Thus, the present study investigated the role of non-invasive biomarkers (including serum fibronectin) to assess the presence of a severity category of fibrosis *vs* the absence of fibrosis in untreated HCV-infected patients. The overall assessment process involved the correlation of fibronectin, alanine aminotransferase ratio (AAR), aspartate aminotransferase to platelet ratio index (APRI), and fibrosis-4 (FIB-4) with the severity staging performed through SWE. In this study, SWE is considered an alternative gold standard to liver biopsy proven through different existing works[12-19]. This is further validated by calculating diagnostic accuracy measured using standard methods such as sensitivity and specificity, Youden's index, area under receiver operating characteristic curve (AUROC), and likelihood test[20].

Most of the existing studies rely on statistical analysis for evaluation. However, these days artificial intelligence (AI)-based techniques are very popular for analyzing the diagnostic ability of clinical indices in patient-based studies[21]. This is because once the AI model is trained to behave in a certain way using existing diagnostic data, then analyzing the clinical data is far more accurate and faster as compared to standard statistical methods[22]. Researchers from the University of Florida used the data related to HCV recorded in the national HCV registry to train the AI models that were further used to predict various risk factors pertaining to HCV treatment[2]. Thus, we have explored AI-based analysis using a Bayesian Network to validate the diagnostic ability of non-invasive indices for predicting liver fibrosis in HCV patients. Moreover, Bayesian networks reveal the conditional dependence between the non-invasive indices by creating parent-child relationships between them[23]. Furthermore, this method provides a diagnostic range for the values of non-invasive indices that clinicians and medical practitioners can use to know the disease progression and detect the severity of liver fibrosis in HCV patients.

MATERIALS AND METHODS

Subjects

A hospital-based observational study was performed on one hundred indoor (hospitalized) and outdoor (outpatients) adult untreated HCV patients who attended the Department of Medicine, Guru Gobind Singh Medical College and Hospital, Faridkot, Punjab, India. Based on the availability and feasibility of the participants, a non-random convenient sampling technique was adopted. The samples were collected from the Malwa population (prone to insecure use of injections for therapeutic reasons), one of the reasons prevalent in HCV hotspots[5]. The patients who tested positive for HCV-RNA using real-time quantitative Polymerase chain reaction - RTPCR (TracQ-C) assay (with detection limit ≥ 15 IU/mL), were included in this study. The viral load of each patient was recorded. The exclusion criteria were followed and patients with comorbidities were excluded from the study. The HCV diagnosed patients were further recruited according to their LSM category (Table 1) derived from the SWE measurement until the limit of the specific fibrosis category was reached (approximately 25 ± 1 individuals each), after which no more in the category were recruited. All the recruited subjects gave informed consent, regardless of sex or age. The work was undertaken following permission from

Table 1 Liver stiffness measurement cutoffs

Severity of fibrosis	METAVIR stages	LSM in kPa
Normal	F0-F1	2 - 7
Mild fibrosis	F2	7.1-11
Moderate fibrosis	F3	11.1-21
Severe fibrosis	F4	> 21

LSM: Liver stiffness measurement.

the Institutional Ethics Committee established within the University.

Exclusion criteria: The co-infected patients (like hepatitis B or human immunodeficiency virus (HIV), or hepatocellular carcinoma) who were detected as positive using a Triple H card were not considered in the present study. None of the considered subjects was linked to ALT flare (values five-fold the top ceiling normal (45 U/mL) measured using AU480 Beckman Coulter fully automated machine), failed or unreliable liver stiffness calculation (using SWE), having more than one HCV genotype infection, and metabolic dysfunction-associated steatotic liver disease (MASLD). We diagnosed MASLD based on cardiometabolic criteria and ruled out patients with Type 2 diabetes mellitus (+ve), body mass index (≥ 25 kg/m^2), and metabolic abnormalities (such as waist circumference (≥ 94/80 in men/women), blood pressure (≥ 130/85 mmHg), plasma triglycerides (≥ 150 mg/dL), HDL cholesterol [> 40/50 mg/dL for men/women), HOMA-insulin resistance score (≥ 2.5)]. The above test was conducted on an AU480 Beckman Coulter fully automated machine. We used an alcohol use questionnaire to rule out serious alcohol use (> 50/60 g/d for men/women) in the study. Additionally, pregnant women were also excluded from the study. We formalized all the above observations when testing positive for HCV PCR.

Non-invasive indices considered for investigations

The proposed study considered several non-invasive indices for routine and special investigations discussed below.

Routine investigations: All the samples were subjected to routine investigation, including AST, ALT, alkaline phosphatase (ALP), total serum protein (TSP), Albumin, and Bilirubin. These routine investigations were performed on an AU480 automated analyzer (Beckman Coulter). Additionally, a Hematology Analyzer (Erba Elite 580) was used for further routine investigations, including hemoglobin, platelet count, and total leukocyte count (TLC). Beckman Coulter manufactured the reagents used for routine investigations. Lastly, International Normalized Ratio (INR) was derived from the prothrombin time (PT) test performed with the Phosphoplastin RL reagent. INR was calculated based on the formula. INR = Patient PT ÷ Control PT.

Special investigations: Fibrosis-4 (FIB-4): Developed in the APRICOT study by Sterling *et al*[10] to predict fibrosis and cirrhosis for patients co-infected by HCV/HIV. Eventually, this marker was further validated through several studies concerning HCV patients[24,25]. FIB-4 = [AST (IU/mL) × age (years)]/[platelet count (*109/L) × ALT 1/2 (IU/mL)].

AST to Platelet Ratio Index (APRI): A simple method developed by Wai *et al*[26], calculated using the routine parameters based on the standard formula. APRI = [(AST/upper limit of the normal AST range) × 100]/platelet count.

AST/ALT ratio (AAR): The proportion of AST and ALT concentrations in the blood measured using a blood test.

Plasma fibronectin: Estimated using the Qayee Bio kit on the ELISA Reader. It is based on the double antibody sandwich enzyme-linked immunosorbent method[27].

SWE: This technique was used for the liver stiffness measurement (LSM) using the ultrasound machine Philips Affiniti 70 (USG). SWE is one of the clinical researchers' most popular non-invasive methods for measuring liver stiffness[11].

Procedure and sample collection

A 10 mL venous blood sample was collected after cleaning the venipuncture site with a spirit swab. The sample was then put into a different vacutainer depending on the type of test and allowed to clot, and then the serum was separated by centrifugation and analyzed. Ethylenediaminetetraacetic acid tubes-BD Vacutainer was used for hematology tests (Hb, TLC and platelet count), sodium citrate vacutainer for PT test, plain red vacutainer for TSP, albumin, bilirubin, AST, ALT, and ALP assays. The viral markers were performed first, and then HCV RNA by reverse transcriptase - polymerase chain reaction was performed and estimated from a Nationally Accredited Laboratory recognized and approved by the Punjab Government. The LSM has been performed using SWE. The USG machine (Philips Affiniti 70) was used to visualize the right lobe of the liver (from the intercostal space) with patients lying with their right arm in maximum abduction while in the supine position. The patients were asked to hold their breath for approximately 5 s to perform imaging. With the visual depth of the system set at 8 cm visual depth, the region of interest was fixed at 1-2 cm below the right liver capsule, with intra-hepatic vessels and gallbladder at a distance apart. The system was calibrated to adjust the sample volume depth at 4 cm or under. The stiffness of the liver was computed automatically by the calibrated system, and the results were generated as the velocity of the shear wave (represented as vs-m/s). Further, the mean elastic modulus (in kPa) was calculated automatically inside the region of interest. The specific segment of the liver was shot 10-12 times, with average result reliability considered with ten successful shots, and the measurement success rate of > 80% was acquired.

In this study, the proposed cutoffs for LSM are described according to the degree of fibrosis in reference to METAVIR stages shown in Table 1. For a diagnostic test, the cutoff value is not universal and is different based on the region, machine and disease condition[28]. So, the cutoff used in the present study was determined according to the USG machine and the range provided for that machine by the relevant vendor. We even verified it with some existing studies [12,24] that suggested standardization of these values and fibrosis range calculation.

Statistical and probabilistic analysis

The statistical calculations were performed on the estimated data using SPSS tool version 21 for Windows (IBM Corp., Armonk, NY, United States) to understand the correlation and significance. The results were presented as median ± inter-quartile range (IQR) or mean ± standard deviation. The correlation was calculated for LSM as an ordinal variable. We used the Kolmogorov-Smirnov test to check the normality of data. The comparison of quantitative variables between the study groups was performed using ANOVA-Kruskal-Wallis test.

Then, we calculated the diagnostic accuracy based on sensitivity and specificity, Youden's index, ROC and AUROC, and the Likelihood test. A multiple linear regression analysis of the clinical results followed this. The multi-linear regression was implemented using Python using ordinary least square regression technique. The model calculates the best fit using least squared method. A probability value (*P* value) less than 0.05 was considered statistically significant.

We used a Bayesian network to analyze the data for probabilistic analysis to uncover the complex relationship of non-invasive indices with disease progression. We implemented a Tree-Augmented Na¨ıve Bayesian model in the Genie platform (https://www.bayesfusion.com/). The details of this technique are explained in the following sections.

Tree-augmented Na¨ıve Bayesian network: Bayesian networks, also known as belief networks, are direct acyclical graphs that show the independence of the connection probability distribution over the set of variables[29]. Bayesian networks have several benefits over other machine learning techniques[30], including dealing with uncertainty and incomplete data, incorporating prior knowledge and domain expertise, providing a graphical representation of the relationships between variables, and quickly computing conditional probabilities. Additionally, Bayesian networks are effective for time-series data and dynamic systems, can handle continuous and discrete variables, and produce predictions that are easy to understand. Considering such benefits, we implemented the Tree Augmented Naive Bayes model (TAN), a semi-Naive Bayesian Learning method, to reveal the complex and hidden relation- ship between non-invasive indices for probabilistically detecting liver fibrosis severity in HCV patients. In the TAN model, the class variable (severity) is the parent of all other variables[31]. Among other variables, a parent-child relationship is created by learning from the data depending on the class variable, *i.e.* the severity. The relationships between the variables are classified as below:

Independence: No direct connection between them.

Dependence: Direct relationship with each other.

Conditional dependence: Dependent on each other conditionally.

Diagnostic analysis

Here, we have used four methods to validate the diagnostic accuracy of fibronectin, APRI, and FIB-4. The four methods are discussed below.

Sensitivity and specificity: Sensitivity and specificity are calculated as follows. Sensitivity = TP/(TP + FN). Specificity = TN/(TN + FP). Where, TP = true positive, FN = false negative, TN = true negative, and FP = false positive.

Youden's index: A test is said to have poor diagnostic accuracy if the value of Youden's index (J) equals 0, and a perfect diagnostic test if Youden's index equals 1[20]. It is defined as: J = (Sensitivity + Specificity) – 1.

ROC and AUROC: The AUROC can have a value between 0 and 1, and it acts as a good indicator to depict the goodness of the test[18]. If the curve is closer to the left-upper quadrant and covers a larger area, it tends closer to 1. This shows that the test better discriminates between fibrosis and no fibrosis cases.

Likelihood tests: Two types of likelihood tests were performed: (1) Positive likelihood test (LR+); and (2) Negative likelihood test (LR-). The larger the LR+, the more informative the test. Similarly, the smaller the LR-, the more informative the test. In simple words, LR+ > 10 and LR- < 0.1 are considered good diagnostic test ratios[32,20]. They are defined as: LR+ = sensitivity/(1 − specificity). LR− = (1 − sensitivity)/specificity.

RESULTS

The results obtained after the experimental evaluation and statistical analysis are in the subsequent sections.

Patient characteristics

The present study included 100 HCV Patients, 66 males and 34 females, with a mean age of 42.7± 13 years. According to SWE, based on LSM values, 25 HCV patients were non-fibrotic, while the other 75 patients had liver fibrosis (24 mild, 25 moderate, and 26 severe). We have considered an equal distribution (25 ± 1) across absent, mild, moderate and severe fibrosis categories by considering the patients testing HCV RNA positive jointly with SWE results.

Assessment of biological variables according to degree of fibrosis

Table 2 shows the Median ± IQR of biological variables in all four categories (*i.e.* non-fibrotic, mild, moderate, and severe fibrosis). The *P* value (< 0.05) was considered significant to differentiate the degree of fibrosis among the four categories. In routine investigations, the viral load, AST, ALT, ALP, TLC and platelet count were found to be significant ($P < 0.05$) as median ± IQR was found to be raised with the severity of fibrosis (Table 2). In special investigations, serum fibronectin, APRI, and FIB-4 were considered significant ($P < 0.05$) in differentiating the degree of fibrosis across the four study groups (no fibrotic, mild, moderate, and severe fibrosis).

Correlation and significance

We have calculated Pearson's correlation coefficient (R)[33] and the significance of investigations concerning LSM.

Routine investigations: The correlation coefficients and significance with regard to the routine investigations concerning LSM are shown in Table 3. It can be seen that viral load, AST, ALT, ALP, TLC, and platelet count are highly significant ($P < 0.05$). At the same time, all other indices are insignificant ($P > 0.05$) according to the obtained results (Table 3).

Special investigations: Table 3 shows the correlation and significance concerning special investigations for all cases with respect to LSM.

Serum fibronectin levels: The validity assessment of fibronectin for predicting fibrosis was also done through correlation. Table 3 depicts a high correlation ($r = 0.929$) for serum fibronectin levels with respect to LSM. A statistically high significance was found, *i.e.* $P < 0.05$.

Fibrotic scores: These indices were validated with respect to LSM based on correlation. The results show that APRI and FIB-4 depict a positive correlation ($r = 0.574$ for APRI and $r = 0.586$ for FIB-4) trend, but AAR depicts a low positive correlation ($r = 0.127$). The results are depicted in Table 3. When compared with LSM values, APRI and FIB-4 were highly significant ($P < 0.05$). However, AAR proved to be non-significant ($P > 0.05$) (Table 3). Figure 1 depicts the correlation plot depicting *r* and *P* value considering special investigations (including fibronectin) *vs* LSM.

Diagnostic accuracy for non-invasive indices

Table 4 compares laboratory investigations for the diagnostic accuracy methods discussed earlier. We want to highlight that sensitivity and specificity are expressed as a decimal and not a percentage in the calculation of Youden's Index. Moreover, Figure 2A-D show the ROC curve of fibronectin, APRI, and FIB-4 in all cases and also in differentiating fibrosis. The cutoffs for fibronectin, APRI and FIB-4, based on which diagnostic analysis was performed, include mild (> 110, > 0.824, > 1.90), moderate (> 140, > 1.19, > 2.45) and severe fibrosis (> 180, > 1.38, > 2.7), respectively. These cutoffs are based on Youden's index.

Linear regression

As we know that correlation just provides the degree to which variables are associated to one another. But, in most of the occasions, just providing simple association is not enough evidence for the overall analysis. So, we used multi-linear regression to explain the variation in one variable, *i.e.* dependent variable with respect to other variables. The multiple linear regression is defined on the basis of the equation. $yi = \beta_0 + \beta_1 x_{i1} + \beta_1 x_{i2} + \ldots + \beta_p x_{ip} + \in$. Where y_i represents the dependent variable, x_i depicts the explanatory variables, β_0 represents y-intercept, β_p represents slope coefficients for each explanatory variable, and $\in$ is the residual.

The variables that we are trying to predict or analyze are called dependent variable as they are dependent on other variables. Here, LSM is considered as the dependent variable. The variables that are considered to analyze or predict the dependent variables are known as explanatory variables. These variables are independent of other variables. Here, the independent variables considered to quantify the relationship include the special investigations, *i.e.* fibronectin, AAR, APRI, and FIB-4. The results obtained from linear regression show that fibronectin ($P < 0.05$) was statistically significant with respect to LSM (dependent variable) as compared to AAR, APRI, and FIB-4. The results obtained using linear regression depicted the p-value of various indices, fibronectin (0.000), AAR (0.430), APRI (0.442), and FIB-4 (0.073). The goodness of the fit (accuracy of the model) comes out to be 0.870 based on R-squared value. Figure 3 depicts the plots showing the regression plot (including the regression equation).

Probabilistic relationship using a bayesian network

The Bayesian Network uses the labeled data for training based on the SWE cutoffs. Once the model is trained, it can automatically detect/predict the relationship of any test data input to the model. In our implementation, we specifically opted for the information-based binning method to determine the cutoffs (known as discretization or binning in Bayesian Networks). This approach ensures that the discretization process is driven by statistical measures, enabling us to identify the most informative cutoff points. By prioritizing information preservation, the information-based binning method offers distinct advantages in accuracy, interpretability, and the ability to capture underlying patterns in the data. Therefore, we believe that using information-based binning for the Bayesian Network cutoffs enhances our approach's reliability and effectiveness. We have constructed a Bayesian graph consisting of nodes and the directed connections between them, where nodes represent the non-invasive indices, and the edges between the two nodes represent potential dependencies between them. The direction of the arrow goes from the influencing variable (parent) to the affected variable (child). Figure 4A shows the dependency network for the Tree-Augmented Na¨ıve Bayesian model implemented in the Genie platform. This dependency network was exploited with all the probabilities for Liver Fibrosis Severity levels ranging from 0-3 (where 0 is no fibrosis), as shown in Figure 4B-E.

Table 2 Biological variables for non-fibrotic, mild fibrotic, moderate fibrotic and severe fibrotic hepatitis C virus cases

Indices	No fibrosis[0]		Mild fibrosis[1]		Moderate fibrosis[2]		Severe fibrosis[3]		*P* value
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	
Routine investigations									
Viral load	112792	56721.5-131470.5	308255	231893.25-424659.75	1050000	696516.5-1334612.5	4956005	3170155.25-9357337	0.000[a]
Hb	13.2	11.35-15	13	11.07-14.7	13	10.65-14	12.95	10.95-13.4	0.674
INR	1	1-1.1	1.1	1.0175-1.3	1.1	1-1.185	1.09	1-1.1775	0.478
TSP	6.9	6.55-7.155	6.9	6.6-7.2	7.1	6.65-7.72	7	6.55-7.2	0.683
Albumin	3.9	3.55-4.5	3.8	3.5-4.175	4.1	3.55-4.3	3.85	3.375-4.3	0.849
Bilirubin	0.68	0.4-0.7	0.7	0.5-0.895	0.7	0.4-0.95	0.8	0.475-1	0.488
AST	43	31.5-63	53.5	30.5-92.75	97	70.5-115.5	108.5	62.5-172.5	0.000[a]
ALT	50	36-77	60	40.25-93.3	92	56-175.5	106.5	85.25-151	0.030[a]
ALP	85	77.5-88	82	78.25-88	102	97.5-131	150	129.5-168	0.000[a]
TLC	8800	7700-9450	8050	6750-9075	7200	6600-8500	7200	6500-7725	0.000[a]
Plt count	216	158-279.5	195	138.75-235	160	111.5-210	130	102-160.25	0.000[a]
Special investigations									
Fibronectin	98	89.82-101.25	118	113.4-126.57	147	138.75-154	204.5	188.975-222.25	0.000[a]
AAR	0.83	0.64-1.08	0.86	0.71-1.21	0.89	0.724-1.26	1.82	1.159-4.37	0.704
APRI	0.52	0.33-0.69	0.6	0.495-1.84	1.67	0.95-2.291	1.82	1.159-4.37	0.000[a]
FIB-4	1	0.63-1.29	1.44	0.98-2.29	2.52	2.04-3.918	3.77	2.68-6.6	0.000[a]

[0]Stage 0, n = 25.
[1]Stage 1, n = 24.
[2]Stage 2, n = 25.
[3]Stage 3, n = 26.
[a]Statistically significant values with cut-off for significance set at < 0.05.
AAR: Alanine aminotransferase ratio; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; APRI: Aspartate aminotransferase to platelet ratio index; AST: Aspartate aminotransferase; FIB-4: Fibrosis-4; Hb: Hemoglobin; INR: International normalized ratio; IQR: Inter-quartile range; Plt: Platelet; TLC: Total leukocyte count; TSP: Total serum protein.

As seen in Figure 4B, when Liver Fibrosis Severity is 0, the probability of serum fibronectin being lower than 150 is 93%. Likewise, the probability of AAR (below 1), APRI (below 2), and FIB-4 (below 2) is likely to be 58%, 90%, and 90%, respectively. Similarly, the probabilistic dependence for other non-invasive indices is also depicted in Figure 4B. Looking at Figure 4E, the liver fibrosis severity is maximum when the probability of serum fibronectin being at maximum values is 52% (with 93% for value above 150). This kind of variation is also visible for FIB-4.

Tables 5 and 6 show the probabilities of Liver Fibrosis severity based on the values of serum fibronectin and a combination of special investigations, namely serum fibronectin, AAR, APRI, and FIB-4. It is seen that there is a direct relation between these non-invasive indices and the severity of Liver Fibrosis. While these indices are at their lowest, the Liver Fibrosis severity tends to decrease. Likewise, when these values increase, the Liver Fibrosis severity also increases.

DISCUSSION

Infection with HCV is a common problem worldwide. Most cases progress to chronic infection with its complications. Due to progressive HCV, liver fibrosis, cirrhosis, liver failure, and hepatocellular carcinoma may occur[34]. In the present study, 100 newly diagnosed HCV patients were considered with a mean age of 42.7 ± 13 years, of which 66% were males and 34% were females. This is found to be similar to other studies that have considered the elderly population in their HCV studies and also have more males as compared to females[12,35,36]. Because treatment of HCV will lead to a reversal of non-invasive markers, only untreated HCV patients were included in our study. Taneja *et al*[37] stated that consuming alcohol > 30 g/d may lead to high fibrosis. So, we have excluded alcoholics (> 80 g/d) from our study. SWE cutoffs were determined using the USG machine 'Philips Affinity 70' (Table 1 depicts the cutoff ranges). Similar cutoffs were defined by Jeong *et al*[12] (F0-F1: 6.77 ± 1.72, F2: 9.98 ± 3.99, F3: 15.8 ± 7.73 and F4: 22.09 ± 10.09).

Table 3 Correlation and significance with respect to liver stiffness measurement

Indices	R	*P* value
Routine investigations		
Viral load	0.663	0.000[a]
Hb	-0.169	0.092
INR	0.041	0.688
TSP	0.081	0.425
Albumin	-0.06	0.552
Bilirubin	0.149	0.14
AST	0.428	0.000[a]
ALT	0.3	0.000[a]
ALP	0.747	0.000[a]
TLC	-0.391	0.000[a]
Plt count	-0.473	0.000[a]
Special investigations		
Serum fibronectin	0.929	0.000[a]
AAR	0.127	0.207
APRI	0.574	0.000[a]
FIB-4	0.586	0.000[a]

[a]Statistically significant values with cut-off for significance set at < 0.05.

AAR: Alanine aminotransferase ratio; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; APRI: Aspartate aminotransferase to platelet ratio index; AST: Aspartate aminotransferase; FIB-4: Fibrosis-4; Hb: Hemoglobin; INR: International normalized ratio; Plt: Platelet; TLC: Total leukocyte count; TSP: Total serum protein.

We have found a strong association between liver fibrosis and liver function tests and routine investigations such as AST, ALT, ALP, platelet count and TLC (Table 2). Fibronectin, APRI, and FIB-4 are found to be highly significant (P < 0.05) according to the degree of fibrosis. Similar findings were found in various existing proposals. For example, Tada *et al*[25] found high significance for platelet count and ALT regarding liver fibrosis (P = 0.003) in HCV cases. Also, Tamaki *et al*[35] found a strong correlation between age and platelet count with liver fibrosis grading (P < 0.05), taking SWE as the main standard method. In the present study, serum fibronectin was highly significant according to the degree of fibrosis (P < 0.05) compared with SWE (Table 2).

Furthermore, compared with SWE, serum fibronectin showed a high positive correlation (r = 0.929; P < 0.05). However, APRI and FIB-4 display a positive correlation but are not as strong as fibronectin (Table 2). Attallah *et al*[38] evaluated the diagnostic value of fibronectin as a predictor of liver fibrosis in patients with chronic HCV infection. Also, they have incorporated a fibronectin discriminant score along with Albumin and APRI, which can decrease the demand for liver biopsy. It was revealed by Yamauchi *et al*[39] that the fibronectin receptor was increased in fibrotic areas and on the plasma membrane of hepatocytes of the fibrotic liver. Also, a positive correlation was obtained between fibronectin and the severity of the liver disease assessed by ALT, AST and serum bilirubin, thus making it suitable to differentiate fibrosis staging in HCV Patients[40]. In our study, AUROC of serum fibronectin, APRI and FIB-4 were calculated as 0.99, 0.67 and 0.725 with cut off of > 110, > 0.82, and > 1.90, respectively, to predict patients with mild liver fibrosis. Our results are similar to Jeong *et al*[12]. They also found low diagnostic accuracy for APRI (0.691) compared to SWE for predicting mild fibrosis. Moreover, Kujur *et al*[24], and Tada *et al*[25] showed mild diagnostic accuracy, *i.e.* (0.842, 0.874) and (0.809, 0.803), respectively, for APRI and FIB-4 in mild fibrosis prediction. In the present study, to detect moderate fibrosis, the AUROC of fibronectin, APRI, and FIB- 4 were calculated as 0.99, 0.83 and 0.853 with a cutoff value of > 140, > 1.19, and > 2.45, respectively. Serum fibronectin showed excellent diagnostic accuracy, while APRI and FIB-4 showed moderate diagnostic accuracy. Our findings are similar to the findings of Kujur *et al*[24], de Oliveira *et al*[41], and Lin *et al*[42]. The AUROC of fibronectin, APRI and FIB-4 were calculated as 0.992, 0.796 and 0.835 with cutoffs of > 180, > 1.38, and > 2.7, respectively, in predicting severe liver fibrosis. Existing studies[41,42] had reported similar results indicating moderate accuracy for APRI and FIB-4 in predicting severe fibrosis. Similar findings for fibronectin were evaluated in[43,44] showing that the level of fibronectin increases significantly with the progression of fibrosis staging. Moreover, Ghafar *et al*[43] showed that fibronectin had a 65% sensitivity with a cutoff of 85.6 and stated that fibronectin is associated with significant fibrosis. A retrospective study conducted by Cassinotto *et al*[45] stated that with SWE as a reference method, FIB-4 had a sensitivity, specificity, and AUROC as 71.4%, 91.4%, and 0.837, respectively. A recent study conducted by Thanapirom *et al*[46] to assess liver fibrosis using non-invasive markers. In this study, APRI and FIB-4 correlated well with SWE. Using magnetic

Table 4 Diagnostic accuracy for fibronectin, aspartate aminotransferase to platelet ratio index, and fibrosis-4

Indices	S. Fibronectin, %	APRI, %	FIB-4, %
Fibrosis			
Sensitivity	98.70	73.30	73.30
Specificity	100	88	96
Youdens index	0.987	0.613	0.693
AUROC	1	0.829	0.85
LR+	> 10	< 10	> 10
LR-	0.013	0.303	0.277
Mild fibrosis			
Sensitivity	100	45.80	45.80
Specificity	96	88	96
Youdens index	0.96	0.338	0.418
AUROC	0.999	0.672	0.725
LR+	> 10	< 10	> 10
LR-	0	0.615	0.564
Moderate fibrosis			
Sensitivity	92.20	80.40	82.40
Specificity	100	77.60	85.70
Youdens index	0.922	0.579	0.681
AUROC	0.993	0.836	0.853
LR+	> 10	< 10	< 10
LR-	0.0784	0.252	0.205
Severe fibrosis			
Sensitivity	96.20	84.60	92.30
Specificity	98.60	62.20	71.60
Youdens index	0.948	0.468	0.639
AUROC	0.922	0.796	0.835
LR+	> 10	< 10	< 10
LR-	0.038	0.247	0.107

APRI: Aspartate aminotransferase to platelet ratio index; AUROC: Area under receiver operating characteristic curve; FIB-4: Fibrosis-4; LR+: Positive likelihood test; LR-: Negative likelihood test. Some of the values are converted into percentages. Cutoffs: Mild (> 110, > 0.824, > 1.90), moderate (> 140, > 1.19, > 2.45) and severe fibrosis (> 180, > 1.38, > 2.7), respectively.

resonance elastography as a reference method, the diagnostic performance of SWE, APRI and FIB-4 was 0.87, 0.83, and 0.79 in differentiating mild fibrosis, and 0.96, 0.89, and 0.91 for cirrhosis, respectively.

In the present study, we have analyzed the diagnostic accuracy to validate the effectiveness of the performed tests for discriminating the patients according to fibrosis staging. Looking at sensitivity and specificity, it is evident that serum fibronectin shows high diagnostic accuracy in discriminating between non-fibrosis, mild, moderate, and severe fibrosis cases. However, APRI and FIB-4 show different variations. They depict lower values for mild fibrosis, which increase as the disease severity increases (Table 3). Thus, APRI and FIB-4 do not strongly discriminate between different stages of fibrosis severity. Youden's Index also depicts a similar trend and advocates fibronectin as a strong diagnostic predictor, with results ranging between 92.2% and 98.7% for mild to severe fibrosis cases (Table 3). Finally, the likelihood tests also support fibronectin as a strong diagnostic marker for predicting liver fibrosis for HCV patients (Table 3). Additionally, increased levels of fibronectin are reported in patients with fibronectin glomerulopathy (autosomal disease), Duchenne muscular dystrophy, rheumatoid vasculitis, preeclampsia, and collagen vascular diseases[47]. In the present study, we affirmed that none of the patients suffered from any other disease that are associated with fibronectin levels. However, we suggest future studies to ensure the validity of this fact with more accuracy. Kim *et al*[47] stated that fibronectin

Table 5 Conditional probability table for liver fibrosis severity with respect to fibronectin

Fibronectin	Fibronectin < 150, %	Fibronectin 150-200, %	Fibronectin > 200, %
LF severity (0)	37	1	1
LF severity (1)	36	1	1
LF severity (2)	25	42	1
LF severity (3)	2	57	98

LF: Liver fibrosis.

Table 6 Conditional probability table for liver fibrosis severity with respect to special investigations

Fibronectin	Fibronectin < 150, %	Fibronectin 150-200, %	Fibronectin > 200, %
AAR	AAR (< 1)	AAR (1)	AAR (> 1)
APRI	APRI (< 2)	APRI (2-3)	APRI (> 3)
FIB-4	FIB-4 (< 2)	FIB-4 (2-4)	FIB-4 (> 4)
LF severity (0)	59	0	0
LF severity (1)	31	0	0
LF severity (2)	8	77	0
LF severity (3)	2	23	100

AAR: Alanine aminotransferase ratio; APRI: Aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis-4; LF: Liver fibrosis.

increases in hepatocellular carcinoma and there is a strong correlation between the liver scarring and fibronectin. The authors mentioned that it may be useful to study fibronectin in the early stages of liver disease. The present study positively correlates with the findings in[47], as the fibronectin has proved to be a valid marker in assessing liver fibrosis.

The present study used a Bayesian Network to understand the hidden relationship between the non-invasive indices. The results depict a strong probabilistic relationship between them in discriminating liver fibrosis stages. The Bayesian Network also provides a trend of the variation in the values of non-invasive indices on liver fibrosis staging. An increase in the values of fibronectin is probabilistically related to an increase in the severity of the disease. Thus, we could predict different diagnostic ranges (cutoffs) for the investigations in relation to the different stages of disease progression (Tables 5 and 6). These cutoffs can be used by clinicians to interpret the results obtained in their equivalent local setting. This may vary if local settings are not standardized and the AI model is trained using totally varied data. It would be suitable to use these cutoffs in conjunction with positive HCV PCR to handle this issue. As visible from Table 5, if we rely just on fibronectin as a diagnostic test, there is a 98% probability that serum fibronectin (> 200) is related to patients with severe fibrosis. This is in line with the statistical findings that suggest fibronectin value > 180 is associated with severe fibrosis. If the fibronectin value is below 150, there is a 37% chance of no fibrosis, a 36% chance of mild fibrosis, and 25% chance of moderate fibrosis, and just 2% chance of severe fibrosis. This means a fibronectin value below 150 is not related to severe fibrosis, as depicted by the AUROC. Similarly, a fibronectin value between 150-200 shows a 42% probability of moderate fibrosis and a 57% probability of severe fibrosis. Table 6 depicts the results where Bayesian Network considers data corresponding to all four special investigations. It shows that if fibronectin (>200), AAR (>1), APRI (>3), and FIB-4 (>4), then there is 100% chance of severe fibrosis. These findings validate the findings based on AUROC and statistical analysis. But, we suggest this aspect needs further validation as this is the first study that suggests diagnostic cutoffs using AI. The accuracy for the Bayesian network was just above 90%, so a larger data size (with high data quality) can be used in the future to provide a strong validation to support the findings of this work.

CONCLUSION

We concluded that serum fibronectin is a strong non-invasive marker in predicting and differentiating the degree of fibrosis among HCV patients. This conclusion is supported by several diagnostic validators, all of them supporting the role of fibronectin as a potential alternative for diagnosing liver fibrosis in the HCV population. Moreover, this study also includes a timely and novel AI technique known as Bayesian Network to understand and uncover the hidden relationship between various routine and special investigations conducted for detecting liver fibrosis in HCV patients. It depicts a strong relationship between fibronectin and liver fibrosis severity stages. This study also analyses the trend of variation of values of routine and special investigations to uncover the tentative range that can be used by diagnostics

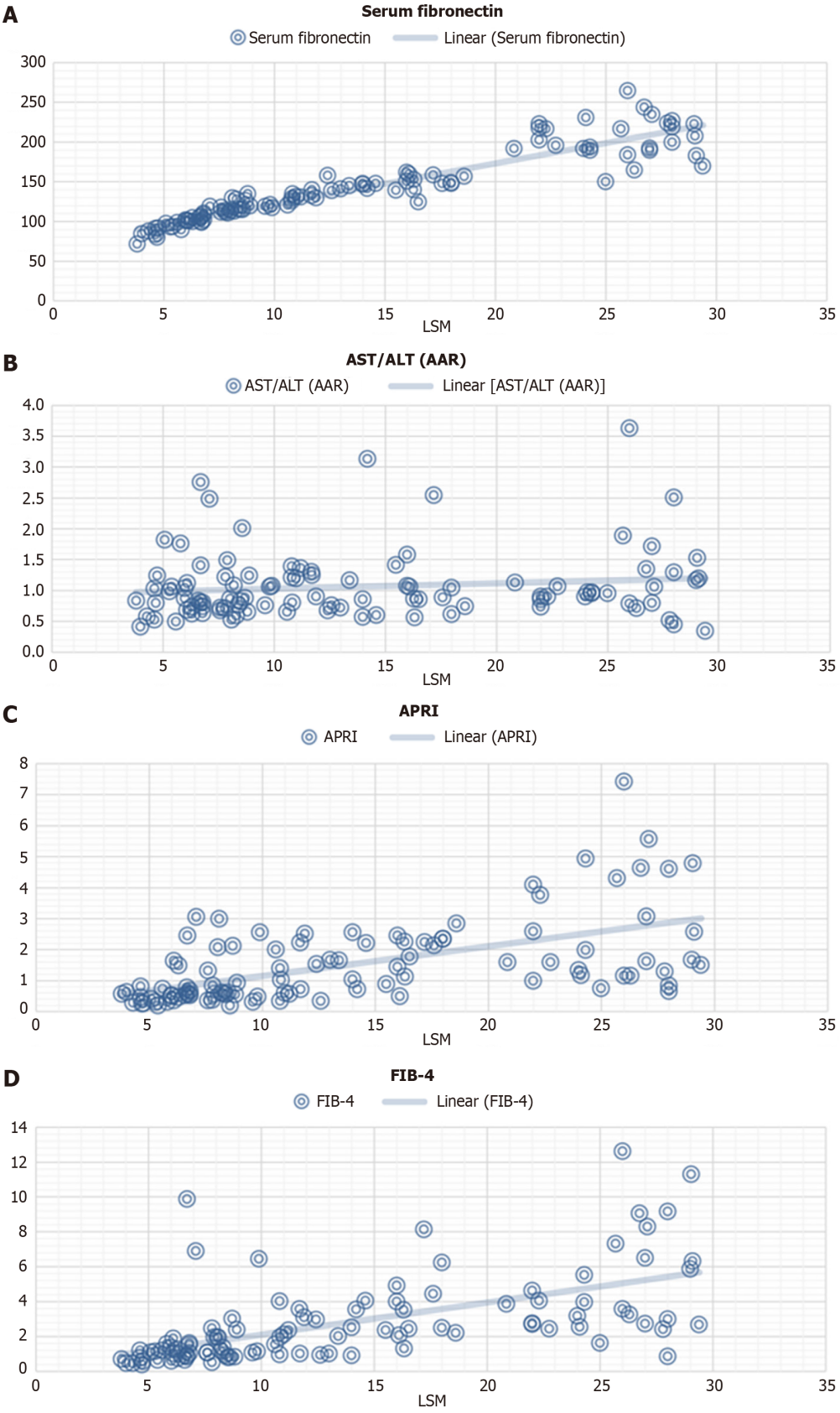

Figure 1 Correlation plots for special investigations *vs* liver stiffness measurement. A: Serum fibronectin *vs* liver stiffness measurement (LSM); B: Alanine aminotransferase ratio (AAR) *vs* LSM; C: Aspartate aminotransferase to platelet ratio index (APRI) *vs* LSM; D: Fibrosis-4 (FIB-4) *vs* LSM. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

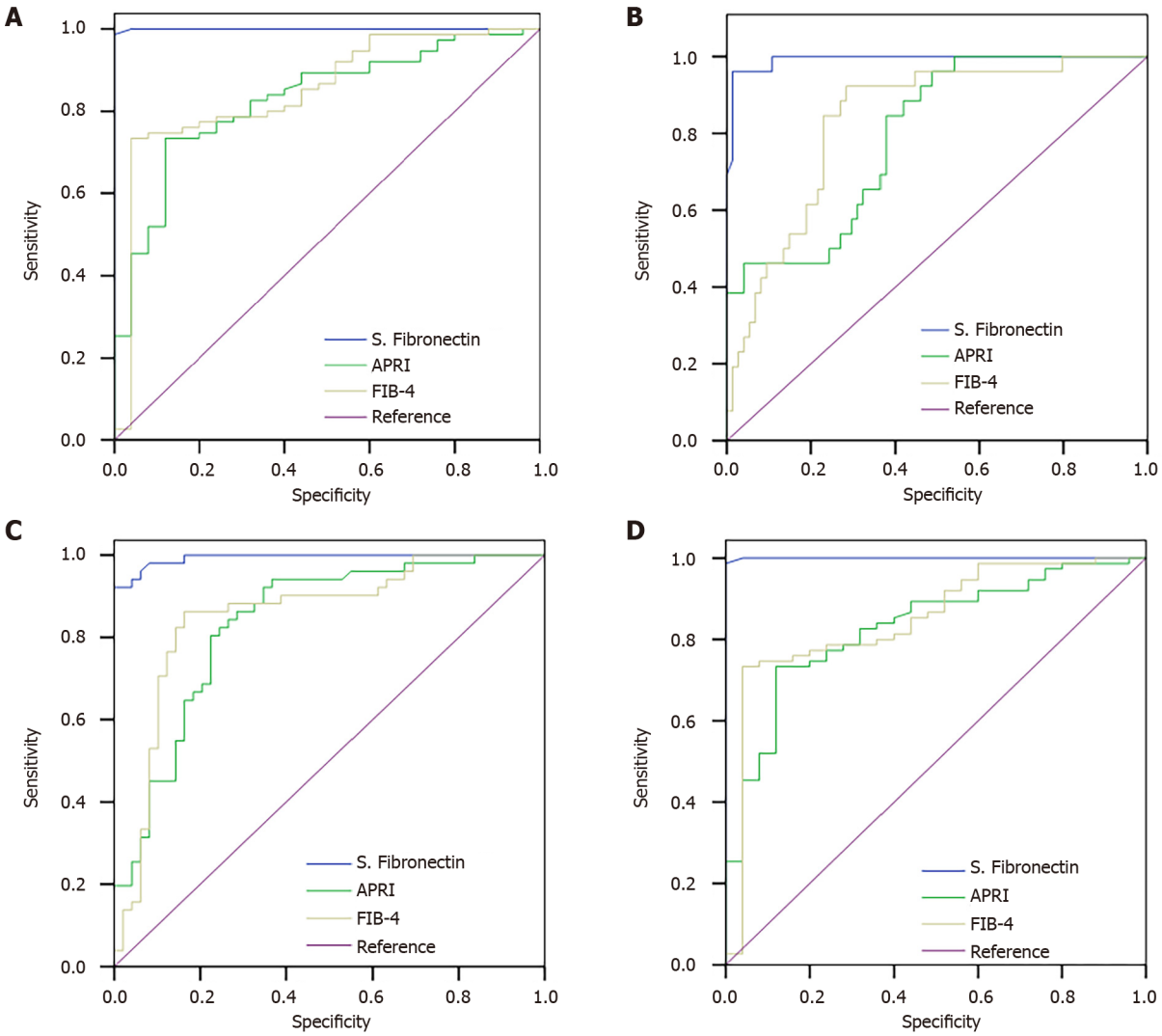

Figure 2 Receiver operating characteristic curves. A: Fibrosis; B: Severe fibrosis; C: Moderate fibrosis; D: Mild fibrosis. S. Fibronectin: Serum fibronectin.

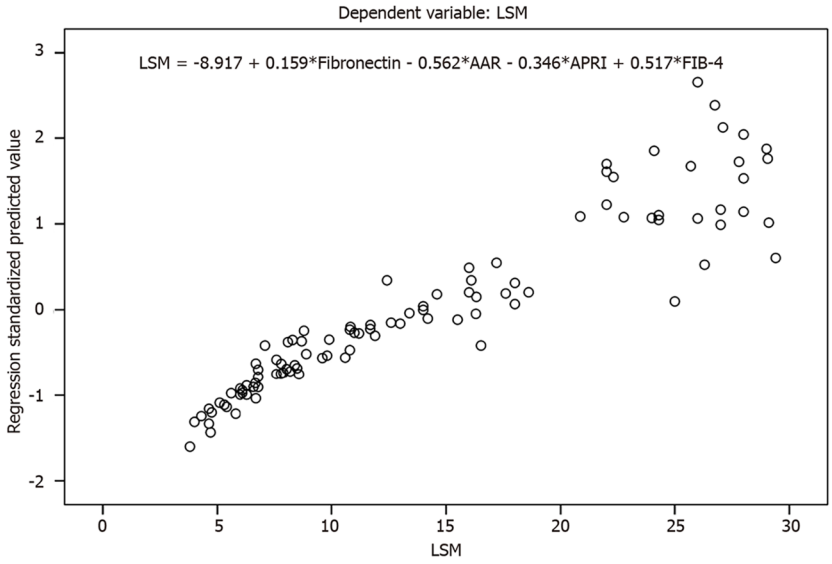

Figure 3 Regression plot.

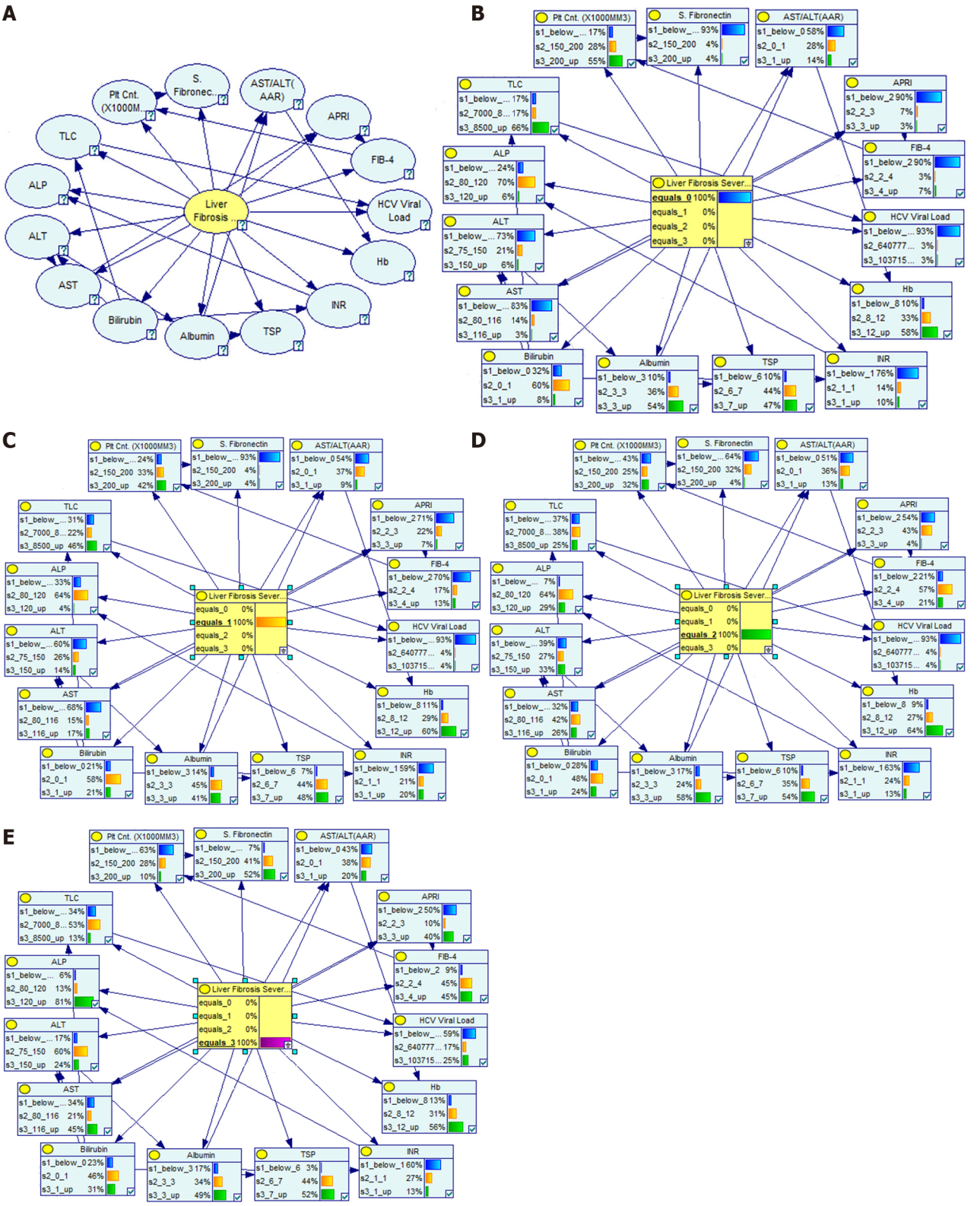

Figure 4 Bayesian dependency plots. A: Dependency network for non-invasive indices and liver fibrosis created using tree-augmented naïve Bayesian technique; B: Fibrosis; C: Severe fibrosis; D: Moderate fibrosis; E: Mild fibrosis.

and clinicians for further studies or for detecting fibrosis stages in HCV patients. However, it is suggested that further studies considering larger sample size and demographic diversity should be conducted.

FOOTNOTES

Author contributions: Kaur N designed the research study, performed the research, contributed to the statistical and analytical analysis and did constructive writing along with main revision of the study; Goyal G conceptualized the study, guided in research and paper writing; Garg R and Tapasvi C guided in research and gave inputs in writing; Demirbaga U performed data analysis using machine learning and contributed to paper writing; All authors have read and finalized the manuscript.

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Country/Territory of origin: India

ORCID number: Navneet Kaur 0000-0002-1766-1636; Gitanjali Goyal 0000-0002-2105-1312; Ravinder Garg 0000-0003-0916-2412; Chaitanya Tapasvi 0000-0002-0244-1200; Umit Demirbaga 0000-0001-5159-0723.

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ORIGINAL ARTICLE

Observational Study

Violence study of healthcare workers and systems in the Caribbean: ViSHWaS-Caribbean study

Reshon Hadmon, Daniella Myriam Pierre, Akshat Banga, Jacques W Clerville, Hans Mautong, Precious Akinsanya, Rajat Das Gupta, Sama Soliman, Tolupe M Hunjah, Bamidele A Hunjah, Hafeez Hamza, Ruman Khurshid Qasba, Faisal A Nawaz, Salim Surani, Rahul Kashyap

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Reshon Hadmon, Department of Medicine, St. George University School of Medicine, Grenada FZ818, West Indies

Daniella Myriam Pierre, Faisal A Nawaz, Salim Surani, Rahul Kashyap, Department of Research, Global Remote Research Scholar Program, St Paul, MN 55101, United States

Daniella Myriam Pierre, Department of Public Health, Cuny Graduate School of Public Health and Health Policy, New York, NY 10027, United States

Akshat Banga, Department of Internal Medicine, Sawai Man Singh Medical College, Jaipur 302004, India

Jacques W Clerville, School of Public Health, Georgia State University, Atlanta, GA 30302, United States

Hans Mautong, Department of Sociology and Demography, School of Health, Universidad de Especialidades Espiritu Santo, Samborondon 092301, Ecuador

Precious Akinsanya, Department of Medicine, Holy Name Medical Center, Teaneck, NJ 07666, United States

Rajat Das Gupta, Department of Epidemiology and Biostatistics, University of South Carolina, Columbia, SC 29208, United States

Sama Soliman, Department of Medicine, Pavlov First State Medical University of St Petersburg, Sankt-Peterburg 197022, Russia

Tolupe M Hunjah, Department of Medicine, University of Suffolk, Ipswich IP4 1QJ, United Kingdom

Bamidele A Hunjah, Department of Medicine, Unicaf University of Zambia, Lusaka 20842, Zambia

Hafeez Hamza, Department of Pharmacy, School of Pharmacy, Girne 99428, Cyprus

Ruman Khurshid Qasba, Department of Medicine, Sher-i-Kashmir, Institute of Medical Science, Srinagar 190011, India

Faisal A Nawaz, Department of Psychiatry, Al Amal Psychiatric Hospital, Emirates Health

Services, Dubai, United Arab Emirates

Salim Surani, Department of Medicine and Pharmacology, Texas A and M University, College Station, TX 77843, United States

Salim Surani, Rahul Kashyap, Anesthesiology and Critical Care, Mayo Clinic, Rochester, MN 55905, United States

Rahul Kashyap, Department of Research, Wellspan Health, York, PA 17403, United States

Co-first authors: Reshon Hadmon and Daniella Myriam Pierre.

Corresponding author: Salim Surani, FCCP, MD, Professor, Department of Medicine and Pharmacology, Texas A and M University, College Station, 40 Bizzell Street, TX 77843, United States. srsurani@hotmail.com

Abstract

BACKGROUND

Violence against healthcare workers (HCWs) in the Caribbean continues to prevail yet remains underreported. Our aim is to determine the cause, traits, and consequences of violence on HCWs in the Caribbean.

AIM

To determine the cause, traits, and consequences of violence on HCWs in the Caribbean.

METHODS

This research adopted an online cross-sectional survey approach, spanning over eight weeks (between June 6th and August 9th, 2022). The survey was generated using Research Electronic Data Capture forms and followed a snowballing strategy to contact individuals using emails, social media, text messages, *etc.* Logistic regression analysis was performed to evaluate the variables that influence violence, including gender, age, years of experience, institution type, and night shift frequency.

RESULTS

The survey was completed by 225 HCWs. Females comprised 61%. Over 51% of respondents belonged to the 21 to 35 age group. Dominica (n = 61), Haiti (n = 50), and Grenada (n = 31) had the most responses. Most HCWs (49%) worked for government academic institutions, followed by community hospitals (23%). Medical students (32%), followed by attending physicians (22%), and others (16%) comprised the most common cadre of respondents. About 39% of the participants reported experiencing violence themselves, and 18% reported violence against colleague(s). Verbal violence (48%), emotional abuse (24%), and physical misconduct (14%) were the most common types of violence. Nearly 63% of respondents identified patients or their relatives as the most frequent aggressors. Univariate logistic regression analyses demonstrated that female gender (OR = 2.08; 95%CI: 1.16-3.76, P = 0.014) and higher frequency of night shifts (OR = 2.22; 95%CI: 1.08-4.58, P = 0.030) were associated with significantly higher odds of experiencing violence. More than 50% of HCWs felt less motivated and had decreased job satisfaction post-violent conduct.

CONCLUSION

A large proportion of HCWS in the Caribbean are exposed to violence, yet the phenomenon remains underreported. As a result, HCWs' job satisfaction has diminished.

Key Words: ViSHWaS; Healthcare workers; Violence; Survey; Workplace violence; Caribbean; Cross-sectional study

Core Tip: The ViSHWaS-Caribbean study followed the guiding principles from the ViSHWaS global study to identify the probable risk factors, characteristics, and outcomes of violence on Caribbean healthcare workers (HCWs). The results were in line with previous studies carried out worldwide and showed that a large proportion of Caribbean HCWs were exposed to violence, leading to job dissatisfaction. The solution to this problem would be to conduct longitudinal analysis/research. Stakeholders should enact regulatory changes to lessen this dispute, and social activities are necessary to strengthen the bonds between HCWs and the communities they serve.

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INTRODUCTION

Healthcare workers (HCWs) face an increased risk of violence worldwide, as per the Pan American Health Organization [1]. However, workplace violence (WPV) in healthcare remains largely tolerated, underreported, and ignored[2]. Although the definition of WPV varies between organizations, nonetheless, it is generally agreed to be both physical and non-physical, inclusive of physical assault, verbal abuse, bullying, intimidation, sexual harassment, and any threatening disruptive behavior at the workplace[3]. According to the World Health Organization, about 62% of HCWs have experienced WPV at least once, with verbal abuse being the most common form of non-physical violence, followed by threats and sexual harassment[4].

Numerous studies have been done to quantify the problem, describing the incidence, prevalence, and impact of WPV among HCWs[5,6]. Such studies have led to reforms in regional guidelines and task forces to mitigate the effect of this widespread quagmire[7,8]. However, the paucity of similar studies in developing countries, including those in the Caribbean, creates a vacuum in measures to understand violence against HCWs, its impact on the healthcare sector, and possible mitigation strategies in these regions as well as on a global scale. Thus, the Violence in HCWs and Systems (Vishwas) study was conducted in 110 countries, including countries that make up the Caribbean Islands, to evaluate the global frequency, cause, and outcomes of violence in the healthcare sector field[9].

A 2016 cross-sectional study in Barbados reported that 63% of the nursing and physician respondents experienced at least one episode of violence within one year of the study, with verbal abuse (63%) reported as the most common form, followed by bullying (19%), and sexual harassment (7%)[10]. Patients were reported as the main perpetrators of the violence. Female gender and nurses were more likely to experience violence compared to males and physicians[10]. A single-center study on lateral violence among nurses in a Jamaican hospital reported exposure to lateral violence in 96% of respondents, with 7% rating the exposure as moderate to severe. Respondents stated that lateral violence created a hostile workplace environment, with half of the nurses surveyed sharing an intent to resign[11]. In 2022, two physicians were abducted in Haiti, allegedly due to gang violence, leading to the closure of four hospitals in Haiti in a protest against the increasing vulnerability of HCWs in the country[12]. Though the numbers reported by the few studies conducted in the Caribbean seem egregious, it is essential to note that violence against HCWs remains underreported. Moreover, the few studies conducted in Caribbean countries either focused on one subset of HCWs (*e.g.*, nurses) or were limited to one institution.

This ViSHWaS-Caribbean is a cross-sectional study that aims to understand the risk factors, characteristics, and impact of violence experienced by HCWs in the Caribbean and identify the causal agents and mitigation strategies.

MATERIALS AND METHODS

A detailed, step-by-step description of our research methodology is provided in our previous works[9,13]. An overview of the ViSHWaS-Caribbean cross-sectional study methodology is provided in the following sections.

Study design and sampling strategy

A cross-sectional survey-based observational study was designed to investigate the burden of HCW-related violence in the Caribbean. The study was part of the ViSHWaS global study[9]. The online survey was generated using Research Electronic Data Capture forms.

The ViSHWaS-Caribbean study utilized the core competencies of the Global Remote Research Scholar Program in human subject-based research, global team dynamics, and data collection, analysis, and interpretation and expanded upon the field. The investigators comprised of a core team and country/regional collaborators. The core team met bimonthly to track the progress and discuss strategies to improve participant recruitment.

Survey distribution strategy

To maximize the number of responses, a "hub and spoke model" of team building was implemented[14]. A snowball sampling technique was utilized to disseminate the survey through in-person meetings, text messages, emails, and various social media platforms, including LinkedIn, WhatsApp, and X, between June 6th and August 9th, 2022[13].

Various promotional YouTube videos were recorded to achieve a larger viewership, and Spanish and Arabic voice translations of the survey were recorded to cater to a larger audience. Following an eight-week period, 225 unique responses from seven Caribbean countries were collected and later analyzed.

Data source

The study adopted a convenience sampling methodology for selecting potential participants from Cuba, Dominica, Dominican Republic, Grenada, Haiti, Jamaica, and Trinidad and Tobago (Figure 1).

Statistical analysis

Descriptive statistics were used to summarize the data. Categorical variables, such as the burden of violence and its impact on HCWs, were estimated using percentages. All statistical analyses were performed with Stata software (version 17.0SE, StataCorp). We used simple and multiple logistic regressions for our univariate and multivariate analysis, respectively, to determine potential WPV predictors. The impact of major (independent) and secondary factors on the likelihood of HCW violence was investigated using univariate models. Multivariate-adjusted models were created concurrently to account for the confounding variables. The models' collinearity was introduced by the years of experience and age of the HCWs. Removing the years of experience from the modified models was the solution. Lastly, we used a χ^2 test to evaluate the relationships between the gender of HCWs and the four distinct violence subtypes. Regardless of statistical significance, all relevant variables were considered for the multivariate model. A P-value < 0.05 was significant.

Ethical considerations and associated publications

The ViSHWaS study was granted an exemption from the Mayo Clinic Institutional Review Board. Various subsets of the ViSHWaS manuscript[9] have been accepted and published as abstracts for presentation at several regional and international conferences, and in preprints.

RESULTS

Socio-demographic characteristics of study participants

Out of a total of 225 HCWs, 61% were females, 51% were in the age category of 26-36, and about 44.4% were mixed race (Table 1). Among the seven Caribbean countries (n = 225), most participants came from Dominica (27.1%), followed by Haiti (22.2%), Grenada (13.8%) Dominican Republic (13.8%), Cuba (11.6%), Trinidad and Tobago (6.2%), and lastly Jamaica (5.3%) (Figure 1). Physicians in training formed 46.7% of the respondents, 21.8% were attending physicians, and 15.6% belonged to "others." Approximately 68% of participants had > 2 years of healthcare work experience. More than half of the respondents worked in government institutions (academic: 48.9%; non-academic: 7.6%), while 22.7% worked in community hospitals, and 11.5% of participants worked in private settings (Table 1).

Violence characteristics

About 39% of the respondents reported experiencing violence themselves, while 17.8% reported violence experienced by their colleague(s). Table 2 highlights that verbal violence (48% of the 225 respondents) was the most common form of violence. Emotional violence was more common amongst "self" respondents (46.6% of 88 *vs* 35.0% of 40), whereas "colleague" respondents reported more physical violence (18.2% of 88 *vs* 37.5% of 40) (Figure 2A).

Among all the HCWs who reported violence against themselves or their colleagues (n = 128), a total of 63.3% of individuals identified patients or their relatives/caregiver/family member as the most frequent aggressors, 14.1% mentioned supervisors, and 14.8% reported encountering more than one type of the aggressor. Nearly half of the respondents (48.4%) felt the frequency of violent incidents to be unaffected by the coronavirus disease 2019 (COVID-19) pandemic, while almost one-third (28.9%) felt an increase in incidence among "self" respondents. A violent episode frequency of once-twice a year was predominant (34/88), while the majority of "colleague" respondents (26/40) reported witnessing an episode more frequently, at one-two per quarter (Table 2).

Based on their perception of relevance, survey participants were asked to rank the listed ten likely reasons for violence (Figure 3). Consistent with the global study, 31.2% of HCWs cited the patient's altered mental state as the most important factor. This was followed by a lack of security for HCWs (11.6%) and a delay in treatment (10.4%). Conversely, the unfulfilled requirements of the patient or their family were regarded as the least important reason by 17.6% of HCWs. A further 12.9% of respondents cited the patient's altered mental state as the least significant factor, while 9.6% pointed to the unexpected prognosis as the least significant factor.

Violence awareness and outcomes

Of the 225 survey respondents, 50.2% confirmed the availability of violence reporting protocols at their institutions, while 35.1% had awareness regarding the Occupational Safety and Health guidelines. Nearly 48.4% of the 128 HCWs who reported experiencing violence had reported the incident to their hospital administration or the police (Table 3).

Out of the 225 survey respondents, 18.7% reported having received training in managing potentially violent conduct, 20% of the respondents felt strongly worried about tackling a violent situation, and 16.4% felt adequately prepared to resolve a potentially violent situation (Figure 2B). Comparably, of the 128 HCWs who reported experiencing violence, 56% lost motivation to work. In contrast, 20% did not let the incident affect them, and another 13% decided to quit their current department, workplace, or profession (Figure 2C) (Table 3).

Univariate and multivariate logistic regression analysis of possible predictors of violence

As shown in Table 4, the univariate logistic regression analyses demonstrated that being female (OR = 2.08; 95%CI: 1.16-3.76, P = 0.014) and working a high frequency of night shifts (OR = 2.22; 95%CI: 1.08-4.58, P = 0.030) were associated with

Table 1 Socio-Demographic characteristics of the ViSHWaS-Caribbean study participants

Demographics	*n* = 225	Percentage (%)
Gender		
Male	81	36.0
Female	137	60.9
Transgender	0	0.0
Gender variant/non-confirming	0	0.0
Other/prefer not to disclose	7	3.1
Skipped	0	0.0
Age (yr)		
18-25	42	18.7
26-35	115	51.1
36-45	53	23.6
46-55	11	4.9
56-65	4	1.8
65+	0	0.0
Skipped	0	0.0
United States-African American	13	5.8
United States-Native Hawaiian/other Pacific Islander	1	0.4
Black-African	65	28.9
South Asian	1	0.4
Hispanic/Latino	81	36.0
Mixed race	17	7.6
Other	42	18.7
Skipped	5	2.2
Type of institution		
Government academic	110	48.9
Government-non-academic	17	7.6
Private academic	21	9.3
Private non-academic	5	2.2
Community hospital	51	22.7
Military hospital	1	0.4
Mission/non-profit hospital	8	3.6
Other	9	4.0
Skipped	3	1.3
Years of experience		
< 1	16	7.1
1 to 2	54	24.0
2 to 5	64	28.4
6 to 10	44	19.6
11 to 20	36	16.0
21 to 30	7	3.1
< 30	1	0.4

Skipped	3	1.3
Work position		
Administration	9	4.0
Nurse practitioner	2	0.9
Attending physician	49	21.8
Auxiliary/support staff	4	1.8
Dentist/dental surgeon	2	0.9
EMT	0	0.0
Fellow in training	10	4.4
Medical student	71	31.6
Occupational therapist	0	0.0
Pharmacist (PharmD)	1	0.4
Physical therapist	3	1.3
Physician assistant	2	0.9
Registered nurse	11	4.9
Researcher	2	0.9
Resident/junior resident in training	24	10.7
Respiratory therapist	0	0.0
Other	35	15.6
Skipped	0	0.0

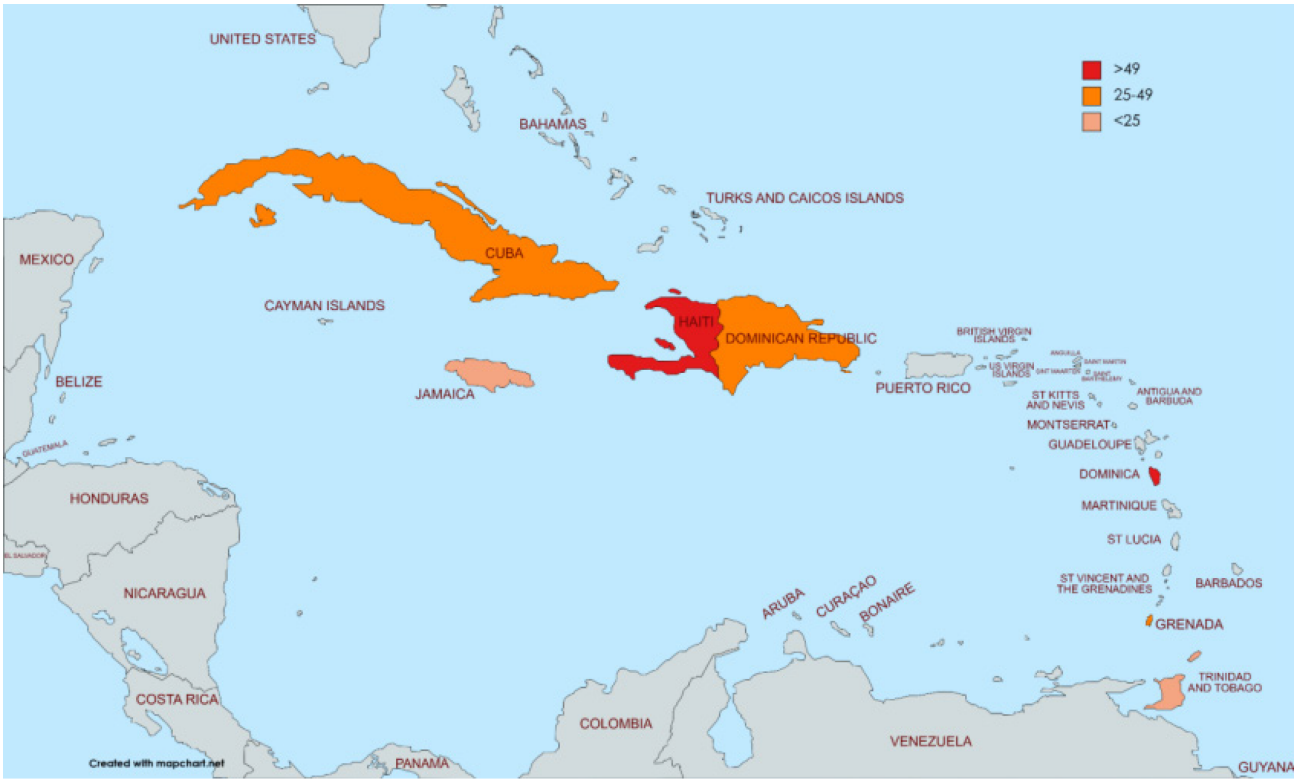

Figure 1 The Map of Participating Countries in the ViSHWaS-Caribbean Study. The color denotes the number of responses from each country.

Table 2 Violence characteristics and attributes

Violence of any form at workplace	Count (*n* = 225)	Percentage (%)
Total yes response - self + colleague (*n* = 225)	128	56.9
Yes response-self (*n* = 225)	88	39.1
Yes response - colleague (*n* =137)	40	17.8
No response - self + colleague (*n* = 225)	97	43.1
Form of violence	Count (*n* = 225)	Percentage (%)
Verbal violence	108	48.0
Emotional violence	55	24.4
Physical violence	31	13.8
Cultural violence	14	6.2
Sexual violence	10	4.4
Online/virtual/cyber harassment	6	2.7
Other	1	0.4
Type of aggressor	Count (*n* =128)	Percentage (%)
More than one type of aggressor	19	14.8
Colleague	9	7.0
Patient	23	18.0
Patient and relative and/or caregiver	8	6.3
Patient and relative and/or caregiver	50	39.1
Supervisor	18	14.1
Frequency of violence; during coronavirus disease 2019 pandemic	Count (*n* =128)	Percentage (%)
Increased	37	28.9
About the same	62	48.4
Decreased	28	21.9
Number of violent episodes in past one year	Survey respondent-self (*n* = 88)	Survey respondent-colleague (*n* = 40)
Every day	1	0
About once a week	8	3
A few times a week	3	0
Once or twice a month	24	10
Once or twice a quarter	18	26
Once or twice a year	34	0

increased odds of having experience violence at the workplace. Amongst various professions, physicians were found to have higher odds of facing violence, with a p-value very close to 0.05 (OR = 4.84; 95%CI: 0.98-23.76, P = 0.052). Whereas work setting, age, and years of experience were not significantly associated with a higher risk of violence.

The same variables were included in the multivariate model to control confounding. It must be noted that years of experience were dropped from the model due to significant collinearity with age. In the multivariate analysis, the 26-35 age group (OR = 0.37; 95%CI: 0.14-0.97, P = 0.043) was the only variable statistically significantly associated with reduced odds of experiencing violence at the workplace. Interestingly, the female gender (OR = 1.84; 95%CI: 0.95-3.59, P = 0.071) and high frequency of night shifts (OR = 1.72; 95%CI: 0.74-3.95, P = 0.205) lost statistical significance and were not associated with increased odds of violence. Similarly, physicians (OR = 4.84; 95%CI: 0.98-23.76, P = 0.088) were not associated with increased odds of violence compared to the reference (Table 4).

DISCUSSION

Violence against HCWs is not a new phenomenon but has emerged as a potential obstacle to efficient healthcare services

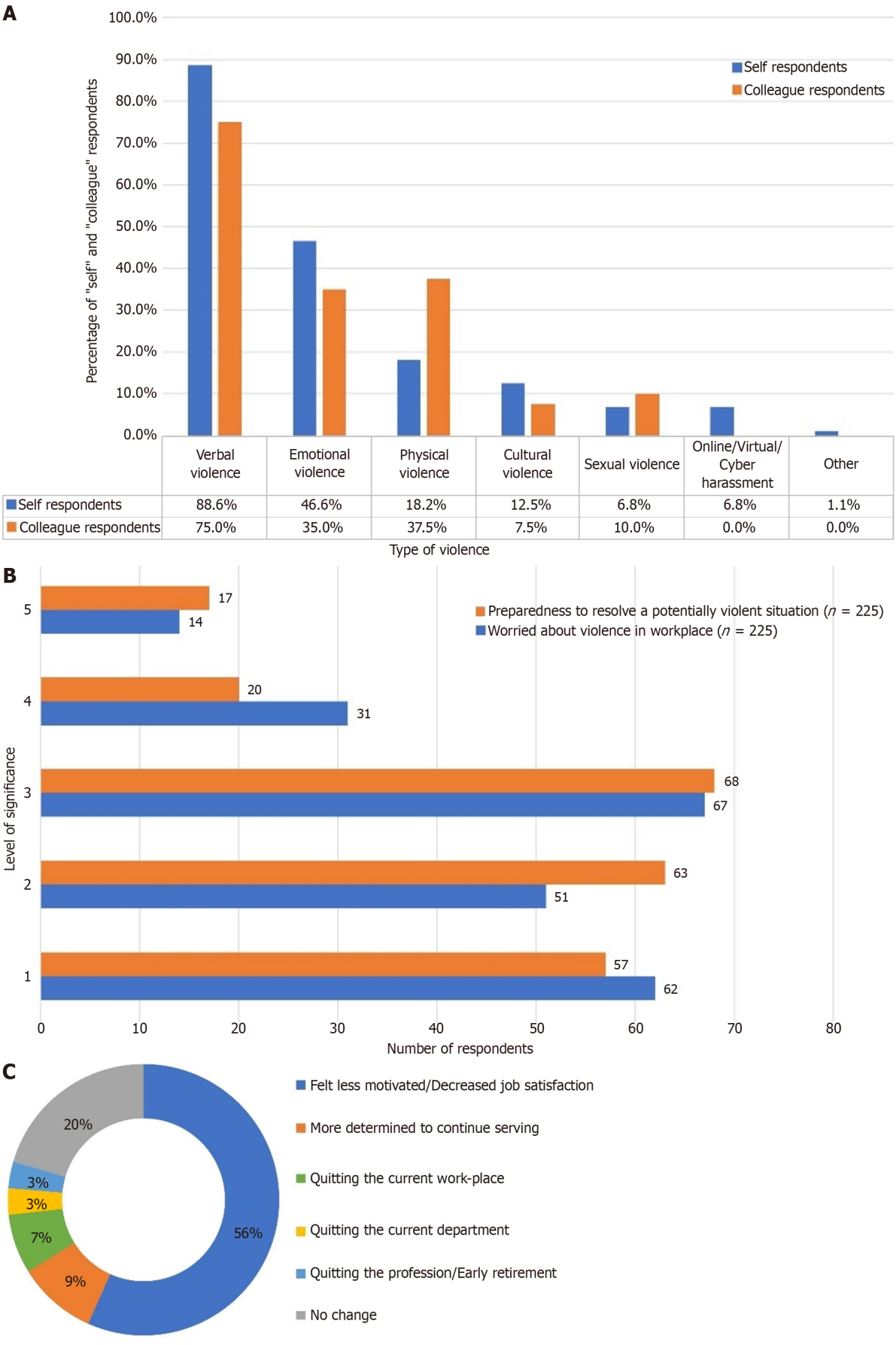

Figure 2 Violence among healthcare workers in the Caribbean. A: Forms of violence amongst "Self" respondents *vs* "Colleague" respondents; B: Tackling a violent situation; C: Outcome of violence amongst healthcare workers.

Table 3 Violence awareness and outcomes

Results	Count	Percentage (%)
Violence incidents reported to the administration or hospital or police (*n* = 128)	62	48.40
Availability of violence reporting procedures at hospital (*n* = 225)	113	50.20
Awareness of occupational safety and health standards (*n* = 225)	79	35.10
Training in violence management (*n* = 225)	42	18.70
Effect of violence on perception of career	Count (*n* = 128)	Percentage (%)
Felt less motivated/decreased job satisfaction	72	56
More determined to continue serving	12	9
Quitting the current work-place	9	7
Quitting the current department	4	3
Quitting the profession/early retirement	4	3
No change	26	20

[15]. Albeit multiple studies in various regions worldwide emphasize this ever-growing subject, the Caribbean islands, like several other developing countries, have had a difficult time addressing this issue[1]. The ViSHWaS-Caribbean study of seven Caribbean island countries adopted established guiding principles from the ViSHWaS global study and found that majority of the results were consistent with those of other comparable studies conducted in various regions worldwide[9].

The following are some important findings of our study: (1) There is a high prevalence of violent attacks among HCWs in various work environments, regardless of their profession, years of experience, and age; (2) in univariate analysis, female gender (OR = 2.08) and working high frequency of night shifts (OR = 2.22) were associated with significantly increased risk of experiencing violence. However, both variables lost statistical significance in the multivariate analysis when controlled for confounders; and (3) HCWs aged 26-35 years were less likely to witness WPV (OR = 0.80).

The ViSHWaS-Caribbean study findings were consistent with the ViSHWaS global study[9] in terms of the probable cause and outcomes of violence against HCWs. However, unlike the global study, being female HCWs in the Caribbean was associated with a higher risk of facing misconduct. In congruence with our results, a study by George *et al*[16] also reported that 64% of women cited violence in terms of verbal abuse and 42% violent threats, and females were more prone to experiencing sexual violence (30%) compared to their male colleagues (4%). They also identified young doctors as being at increased risk of violence[16]. This could be attributed to younger physicians having less experience and expertise in communication and handling problematic situations-Another study by Shahjalal *et al*[17], identified that HCWs working in public healthcare institutions had a higher risk of experiencing physical violence compared to private setups. However, this study reported male HCWs (specifically physicians) of being at higher risk than females, contradicting our findings[17].

The Hospital Safety and Staffing Consumer Survey Report highlighted a few risk factors for violence in healthcare institutions[18]. Staffing shortages, burnout, and mistreatment have emerged as major concerns in managing workload and easing the tensions among staff and patients in the Caribbean. The Pan American Health Organization highlighted that several Caribbean HCWs have migrated due to a lack of respect, work overload, and poor treatment[1]. This has also led to increasing frustration amongst patients and family members, seen through complaints in the media highlighting the dissatisfaction with the healthcare system or response from HCWs. Over time, this phenomenon has evolved into violence against the remaining HCWs in the Caribbean[1,10-12]. Adding to this is the lack of training, experience, and resources in handling such physical and non-physical altercations in healthcare settings[15,19]. The findings of our study indicate that a significant proportion of participants cited a patient's altered state of mind as the most likely factor contributing to incidents of violence against HCWs. This was followed by factors such as inadequate education of the patient or family member, ineffective communication skills when dealing with aggressive patients or family members, insufficient security measures for the HCWs, and treatment delays. On the other hand, unmet care needs of patients or family members, unfulfilled requirements of the patient or their families, and a perception that the assault will be inconsequential for the assailant were considered the least likely explanations. Violence against HCWs can have a detrimental impact on the HCWs, leading to physical hurt, stress disorders, job dissatisfaction and resignation, and even death. This, in turn, damages the healthcare, compromising the patient's wellness[3,19-21].

Addressing violence against HCWs in the Caribbean will require a multidisciplinary team approach[7]. The Crisis Prevention Institute has identified "de-escalation tips" for HCWs when approaching a problematic situation. This enables staff to identify threatening language and any signs of agitation to prevent possible harm to the staff and patients[22].

The ViSHWaS-Caribbean study provided a clear view of the violence experienced in Caribbean countries. After performing the global ViSHWaS survey[9], a snapshot was taken of the Caribbean HCWs' exposure to violence in comparison to other nations. The healthcare systems were reviewed and provided the researchers with information to gather conclusions based on the study objectives. Additionally, using the survey increases the reliability and validity of the findings. Lastly, the data's anonymity was maintained using de-identified data and a web-based survey design.

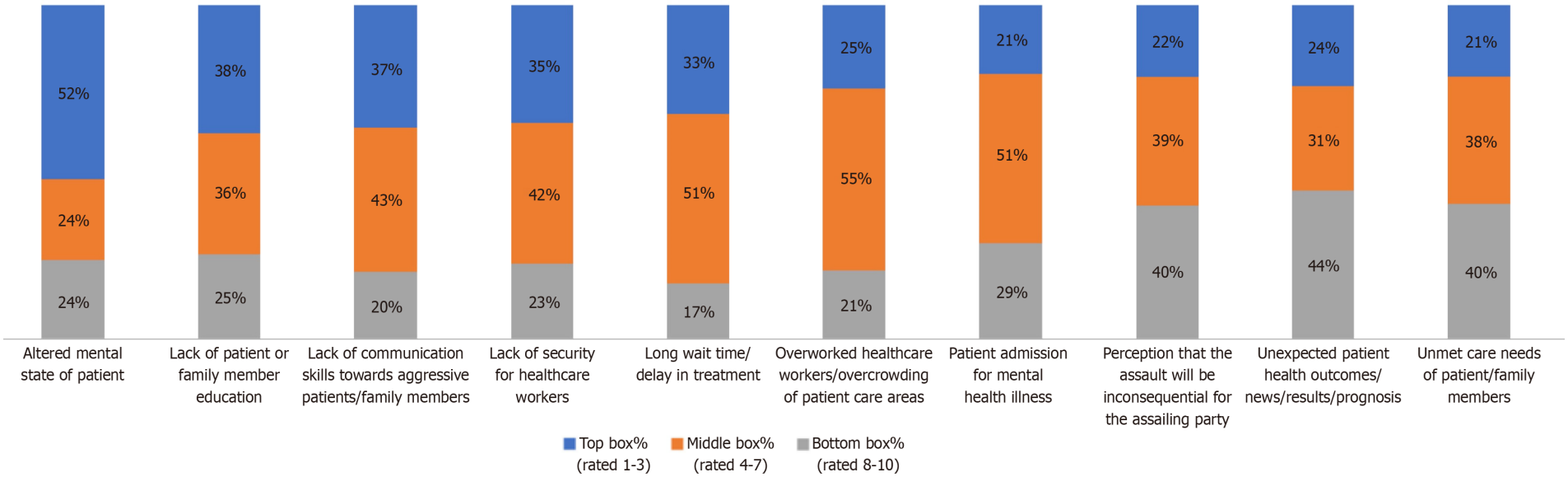

Figure 3 Probable cause of violence among healthcare workers.

It is important to note the limitations of this study. Given that the current research focuses primarily on HCWs, it is possible that this could result in a certain level of bias in the study. Furthermore, response bias poses a concern due to the locations of the self-selected participants and the number of responses with respect to the aggressors of violence among HCWs, which could pose a higher risk of having skewed results. One possible explanation for the skewed results could be the stigma attached to violence in the Caribbean, especially among family and peers. The language was a barrier for a few nations due to the survey being designed in English. The solution to this was for the core team to implement visual and graphical illustrations from educational modes to aid the completion of the surveys. Also, Spanish and Arabic translations were provided *via* recorded messages and peer-to-peer communications; however, translations to languages like French and Creole were unavailable. Because of the cross-sectional design, we were unable to characterize the prevalence of HCW-related violence.

Furthermore, the responses to the 10-point rating questions may contain bias. Because the replies were arranged alphabetically, some respondents may have given the highest priority to the answers that were featured first. Lastly, these results represent a small sample size from the Caribbean region, which limits generalizability to the entire region.

The high patient volume in the Caribbean nations, in addition to a staff shortage, resources, and financial incentives, makes it increasingly stressful for healthcare professionals to work and provide effective care[1]. The multifaceted nature of violence against HCWs makes it an additional stressor[9]. Institutions need to conduct longitudinal research to fully understand the complexities and quantify the scope of this persistent issue[13]. To reduce this disagreement, stakeholders should implement policy measures and social activities are required to improve connections between HCWs and the communities they serve[7].

Table 4 Univariate and multivariate regression analysis for violence estimation

Variable	Univariate					Multivariate				
	OR	Std Err.	95%CI for B		*P* value	OR	Std Err.	95%CI for B		*P* value
			LL	UL				LL	UL	
Gender[1]										
Female	2.08	0.63	1.16	3.76	0.014	1.84	0.63	0.95	3.59	0.071
Work setting[2]										
Public setting	1.44	0.57	0.66	3.13	0.358	1.25	0.56	0.52	2.99	0.618
Other	0.60	0.53	0.11	3.36	0.559	0.79	0.74	0.13	4.92	0.804
Profession[3]										
Medical student	1.89	1.56	0.38	9.5	0.440	1.89	2.29	0.18	20.20	0.598
Nurse	4.00	3.68	0.66	24.30	0.132	5.30	6.50	0.48	58.61	0.173
Physician	4.84	3.93	0.98	23.76	0.052	7.10	8.15	0.75	67.37	0.088
Other HCW	2.14	1.84	0.41	11.42	0.360	3.10	3.65	0.31	31.29	0.337
Age[4]										
26-35	0.80	0.29	0.39	1.63	0.534	0.37	0.18	0.14	0.97	0.043
36-45	0.95	0.40	0.42	2.15	0.90	0.44	0.26	0.14	1.39	0.160
46-55	1.11	0.76	0.29	4.22	0.88	0.42	0.41	0.63	2.82	0.372
Years of experience[5]										
1 to 2	0.93	0.57	0.28	3.10	0.901	-	-	-	-	-
2 to 5	1.61	0.96	0.50	5.16	0.427	-	-	-	-	-
6 to 10	1.52	0.94	0.45	5.14	0.498	-	-	-	-	-
11 to 20	2.20	1.39	0.63	7.62	0.214	-	-	-	-	-
21 to 30	1.65	1.54	0.26	10.31	0.592	-	-	-	-	-
> 30	0.93	0.57	0.28	3.10	0.901	-	-	-	-	-
Night shift frequency[6]										
High	2.22	0.82	1.08	4.56	0.03	1.72	0.73	0.75	3.95	0.20

[1]Reference female category is "male".
[2]Reference work setting category is "private".
[3]Reference profession category is "administrative or researcher".
[4]Reference years of experience category is "18-25".
[5]Reference years of experience category is "< 1".
[6]Reference frequency of night shifts category is "low".
Values are significant at 0.05. HCW: Healthcare workers; Std Err: Standard error; LL: Lower limit; UL: Upper limit.

CONCLUSION

A large proportion of HCWS in the Caribbean were exposed to violence, according to the ViSHWaS-Caribbean online cross-sectional survey. As a result, employee happiness has diminished. Legislative initiatives and interpersonal interactions must be implemented to decrease this discord to boost relationships between HCWs and their communities. More studies should be carried out to understand better the burden of violence against HCWs in the healthcare sector in the Caribbean.

FOOTNOTES

Author contributions: Banga A, Kashyap R, and Mautong H designed research; Hadmon R, Pierre DM, Banga A, Clerville JW, Mautong H, Akinsanya P, Gupta RD, Soliman S, Hunjah TM, Hunjah BA, Hamza H, Qasba RK, Nawaz FA, Surani S, Kashyap R collected the data; Hadmon R, Pierre DM, Banga A, Mautong H, Kashyap R analyzed the data; Hadmon R, Banga A, Kashyap R supervised the paper; Hadmon R, Pierre DM, Banga A, Mautong H, Kashyap R wrote the original draft; Hadmon R, Pierre DM, Banga A, Clerville JW,

Mautong H, Akinsanya P, Gupta RD, Soliman S, Hunjah TM, Hunjah BA, Hamza H, Qasba RK, Nawaz FA, Surani S, Kashyap R reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript. Hadmon R and Pierre DM contributed equally to this work as co-first authors.

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Informed consent statement: The ViSHWaS study was granted an exemption from the Mayo Clinic Institutional Review Board. The consent requirements were waived.

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Data sharing statement: Dataset is available from the corresponding author at srsurani@hotmail.com.

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ORCID number: Reshon Hadmon 0009-0001-7917-9781; Daniella Myriam Pierre 0009-0001-1282-7094; Akshat Banga 0000-0003-2626-8219; Jacques W Clerville 0000-0002-5582-5750; Hans Mautong 0000-0002-4588-6605; Precious Akinsanya 0000-0002-8952-1288; Rajat Das Gupta 0000-0002-7680-676X; Sama Soliman 0000-0002-3275-438X; Tolupe M Hunjah 0009-0005-9271-4522; Bamidele A Hunjah 0009-0006-1304-1188; Hafeez Hamza 0000-0001-9305-9930; Ruman Khurshid Qasba 0009-0001-6637-596X; Faisal A Nawaz 0000-0002-0701-7628; Salim Surani 0000-0001-7105-4266; Rahul Kashyap 0000-0002-4383-3411.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Botulinum toxin type A for treating chronic low back pain: A double blinded randomized control study

Mantu Jain, Shahnawaz Khan, Paulson Varghese, Sujit Kumar Tripathy, Manaswini Mangaraj

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Mantu Jain, Shahnawaz Khan, Paulson Varghese, Sujit Kumar Tripathy, Department of Orthopaedics, All India Institute of Medical Sciences, Bhubaneswar 751019, Odisha, India

Manaswini Mangaraj, Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar 751019, Odisha, India

Corresponding author: Sujit Kumar Tripathy, MS, Professor, Department of Orthopaedics, All India Institute of Medical Sciences, Sijua, Patrapada, Bhubaneswar 751019, Odisha, India. ortho_sujit@aiimsbhubaneswar.edu.in

Abstract

BACKGROUND

Low back pain (LBP) is a prevalent issue that orthopedic surgeons frequently address in the outpatient setting. LBP can arise from various causes, with stiffness in the paraspinal muscles being a notable contributor. The administration of Botulinum toxin type A (BoNT-A) has been found to alleviate back pain by relaxing these stiff muscles. While BoNT-A is approved for use in numerous conditions, a limited number of randomized clinical trials (RCTs) validate its efficacy specifically for treating LBP.

AIM

To study the safety and the efficacy of BoNT-A in minimizing pain and improving functional outcomes in patients of chronic LBP (CLBP).

METHODS

In this RCT, adults aged 18-60 years with mechanical LBP persisting for at least six months were enrolled. Participants were allocated to either the Drug group, receiving 200 Ipsen Units (2 mL) of BoNT-A, or the Control group, which received a 2 mL placebo. Over a 2-month follow-up period, both groups were assessed using the Visual Analog Scale (VAS) for pain intensity and the Oswestry Disability Index (ODI) for disability at the start and conclusion of the study. A decrease in pain by 50% was deemed clinically significant.

RESULTS

The study followed 40 patients for two months, with 20 in each group. A clinically significant reduction in pain was observed in 36 participants. There was a statistically significant decrease in both VAS and ODI scores in the groups at the end of two months. Nonetheless, when comparing the mean score changes, only the

reduction in ODI scores (15 in the placebo group *vs* 16.5 in the drug group, clinically insignificant) was statistically significant (P = 0.012), whereas the change in mean VAS scores was not significant (P = 0.45).

CONCLUSION

The study concludes that BoNT-A does not offer a short-term advantage over placebo in reducing pain or improving LBP scores in CLBP patients.

Key Words: Botulinum toxin type A; Chronic low back pain; Randomized control study; Double-blinded; Pain management; Therapeutic efficacy

Core Tip: This randomized clinical trial investigated Botulinum toxin type A (BoNT-A) for treating chronic low back pain (CLBP) in adults aged 18-60 years old with symptoms persisting for over six months. Participants were divided into two groups: one receiving BoNT-A and the other a placebo, with outcomes measured using the Visual Analog Scale for pain and the Oswestry Disability Index (ODI) for disability. After two months, both groups showed pain reduction, but only the decrease in ODI scores was statistically significant (but clinically insignificant). Ultimately, BoNT-A did not demonstrate a short-term advantage over placebo in reducing pain or improving disability scores in CLBP patients.

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INTRODUCTION

Globally, low back pain (LBP) is a leading cause of pain and disability, significantly impacting individuals' workability worldwide[1]. The influx of LBP cases in the orthopedic outpatient departments of hospitals is on the rise, now representing nearly a third of all consultations. This trend incurs a huge healthcare expenditure. While acute instances of LBP tend to resolve promptly, a notable proportion, between 30%-40%, evolves into chronic LBP (CLBP), characterized by enduring and incapacitating symptoms. CLBP adversely affects individuals' psychosocial, behavioral, and vocational aspects, undermining their productivity and quality of life[2]. Effective management of CLBP is pivotal for diminishing its morbidity and the overall financial strain on healthcare systems[3]. The correlation between the severity of LBP and lumbar stiffness, primarily attributed to the contraction of the erector spinae muscles, highlights the demand for localized muscular interventions. These interventions include physical therapy, rehabilitation exercises, infrared therapy, and Botulinum toxin type A (BoNT-A)[4-7].

BoNT-A has demonstrated efficacy in managing various musculoskeletal conditions, including cervical dystonia, cerebral palsy, and spasticity. Although a few cohort studies have reported its potential advantages for CLBP, the supporting evidence remains limited in quality, signaling an urgent need for further randomized controlled trials (RCTs) to establish more definitive conclusions[8-10]. The current literature comprises only a modest number of RCTs with inconsistent findings[11-13]. Consequently, this study aims to explore the efficacy of BoNT-A in minimizing pain and improving functional outcomes in patients suffering from CLBP, endeavoring to enrich the existing evidence on its therapeutic value.

MATERIALS AND METHODS

Patient recruitment

The study received ethical clearance from the institutional ethics committee (No. T/IM-F/21-22/03) and was officially registered in a clinical trial registry (CTRI/2022/08/044530, https://ctri.nic.in/Clinicaltrials/login.php). It was conducted in the Department of Orthopaedics at our institution, targeting patients with CLBP. Participation was contingent on patients providing written informed consent. The study included adults aged 18-60 years who had been suffering from CLBP for at least six months and were willing to participate. Exclusion criteria were set to exclude individuals presenting with acute low back pain, neuromuscular disorders, or red flags for severe conditions such as malignancy, trauma, tumors, or neurological deficits, all of which were excluded *via* initial X-rays and magnetic resonance imaging. Furthermore, those with radiculopathy, neurogenic claudication, previous back surgery, or known allergies to BoNT-A were not considered for the study.

Sample size

The sample size was estimated based on a previous study by Jazayeri *et al*[14] in 2011. Assuming a difference of 37.5% (50% for the drug group and 12.5% for the control group, with a power of 80% and significance level of 5%, the sample size was calculated to be 40 (*i.e.*, 20 for each group). With a dropout of 20%, a sample of 44 were included in our study:

Total sample = $16 \times [P1 \times (100 - P1) + P2 \times (100 - P2)]/(P1 - P2)^2$.

The participants were allocated to either arm with an allocation ratio of 1:1. The patients and the researcher (who assessed for scoring) were both blinded. Only the person doing the intervention (not involved in collecting scores) opened the sealed envelope and gave the drugs. The patients were divided into 2 groups.

Interventions

Drug arm (Group B): The BoNT-A (Botox, Allergan India private limited) was re-constituted using frozen-dried toxin and mixed with 2 mL of preservative-free 0.9% normal saline to a strength of 100 Ipsen units/mL. The mixture was drawn into a 2 mL syringe fitted with a 22-gauge needle. Four equidistant points on paraspinal muscles, 1.5-2 inches away from the midline, bilaterally, were chosen, and 50 Ipsen units were inserted at each site. Tender or trigger points were preferred, if any. Care was taken to inject the toxin into the muscles' core while avoiding its spillover into the vascular compartment. All injections were performed without electromyographic (EMG) guidance.

Control arm (Group C): A similar dose of normal saline was injected at four points in the paraspinal muscles.

Both the groups received analgesics for not more than seven days, a weekly vitamin D supplement (60 K) for 8 wk, and taught home-based isometric back strengthening exercises to be done twice daily for a period of 15 min.

Outcome measures

Outcome measures for the patients included an evaluation of pain intensity using the visual analog scale (VAS) on a Likert scale from 1 to 10, where "1" represented the least pain and "10" was the worst pain. Additionally, physical impairment and disability were quantified using the Oswestry Disability Index (ODI) questionnaire, with scores out of 100. Assessments were conducted at the initial consultation (baseline) and at the conclusion of the treatment period (eight weeks). At the eight-week follow-up, patients were asked about the duration of pain relief, specifically if pain had recurred. A significant improvement was defined as a greater than 50% change in scores before and after treatment.

Participants were advised to refrain from using opioid medications and from undergoing any other treatments not specified in the study protocol, such as facet joint block injections, throughout the study. The safety profile of the treatment was also monitored by documenting any side effects experienced by the participants.

Statistical analysis

The data was compiled in the Excel sheet. Results were analyzed using SPSS software version 25. The normality testing was done using the Shapiro-Wilk test. The VAS and ODI scores were compared at baseline and at eight weeks using the paired *t*-test. The comparison among different groups was performed using the student *t*-test. A *P* value of less than 0.05 was considered significant.

RESULTS

A total of 44 subjects with CLBP were included in the study. Two patients in each group did not follow up and were excluded. Forty patients were followed up for eight weeks. The details are given in Figure 1. The demographic profile of the patients was found to be similar, as depicted in Table 1. The biochemical parameters are also similar, but Vitamin D deficiency was seen in all patients of both groups (Table 2). The outcome is depicted in Table 3. All the patients had clinical reductions in pain and ODI scores at the last follow-up of eight weeks. A total of 36 patients had significant pain relief. Of these, 17 patients received BoNT-A injections, while the remaining 19 received a placebo. None of the subjects reported any adverse effects due to medication during the course of the study.

Comparison of scores

Both groups had significant reductions in VAS and ODI scores at the end of 8 wk (Table 3). On comparing the change in means in both the groups, it was found that there was a statistically significant reduction in ODI scores (though clinically insignificant). In contrast, the VAS score improvement was insignificant (Table 4).

DISCUSSION

The cause of chronic LBP is multifactorial, but lumbar stiffness due to erector spinal muscle spasm is linked to the level of severity of LBP[5]. This reflex spasm model of back pain has been demonstrated by the EMG readings showing increased activity in the muscles exhibiting pain[15]. BoNT-A has a role as a muscle relaxant, an analgesic, and an anti-inflammatory. The mechanism of action is also diverse, from inhibiting the pain transmitters from the nerve endings and ganglions of peripheral nerves to blocking the release of acetylcholine from the neuromuscular junction[16].

BoNT-A is widely used in the management of many medical and cosmetic issues. The use of BoNT-A in cervical dystonia has yielded great results. Researchers have tried to use in several musculoskeletal ailments (refractory joint pain, tennis elbow, plantar fasciitis) with varying success[17]. Liu[18] tried its use in third transverse process syndrome and

Table 1 Demographic profile of the patients

Variables	Placebo	Botulinum toxin	*P* value
Age (mean ± SD)	39.5 ± 7.55	40.62 ± 9.13	0.67
Male:female	9:11	13:7	
Mean duration of pain (months)	15.20	15.05	0.97
Body mass index (mean ± SD)	23.93 ± 4.23	23.94 ± 3.65	0.99

Table 2 Baseline serum values of biochemical parameters

Variables	Placebo (mean ± SD)	Botulinum toxin (mean ± SD)	*P* value
Serum calcium (mg/dL)	9.29 ± 0.36	9.29 ± 0.65	1.00
Serum alkaline phosphate (IU)	90.1 ± 37.5	81.19 ± 25.20	0.38
Serum phosphate (mg/dL)	3.36 ± 0.61	3.43 ± 0.39	0.66
Serum vitamin D (mg/dL)	8.95 ± 4.15	10.05 ± 3.69	0.38

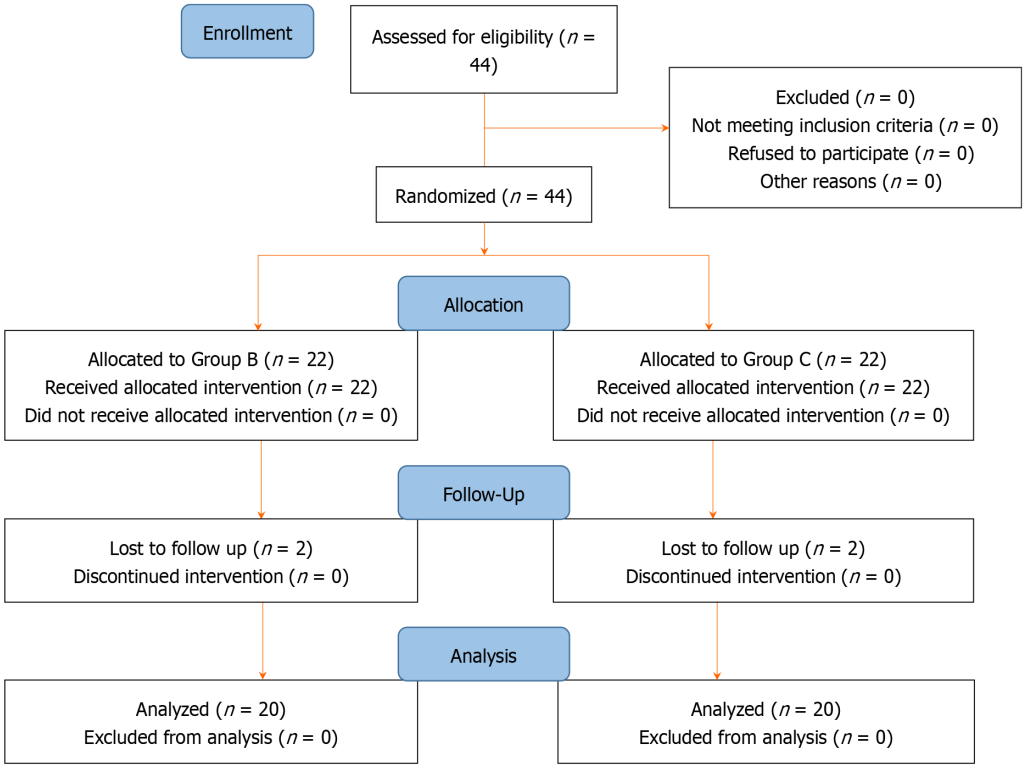

Figure 1 CONSORT diagram of patients.

acupuncture and found it superior[18]. Fishman *et al*[19] successfully used it in treating piriformis syndrome in a 12-wk study[19]. However, De Andrés *et al*[20] compared BoNT-A with saline/bupivacaine in myofascial pain syndrome (iliopsoas and quadratus lumborum muscles) and found no significant difference[20].

Similarly, its use in chronic LBP has been variable. While the open-label trial has clearly shown some reversible beneficial effects in the short term, few RCTs have unpredictable responses, and the final verdict is far from any conclusion. In this further literature review, we chronologically study the outcome pattern in these two different categories of evidence. Jabbari *et al*[8] in 2006, studied 75 patients injected with BoNT-A 200-500 Ipsen units and followed up for 14 months[8]. The authors found 40 (53%) had substantial pain relief at two months, and 90% persisted at the final follow-up. In the same year, Ney *et al*[21] did another study in a cohort of 60 patients of CLBP wherein the authors injected 500 units of BoNT-A[21]. They found that BoNT-A significantly reduced LBP scores in about 58% of patients at the end of 8 wk, which gradually faded away with persistent results at only 16% at four and 8% in six months. Nagarajan *et al*[9] in 2007, conducted a study in Kuwait with eight CLBP subjects injected with 100 units of BoNT-A[9]. The authors found remarkable improvement in pain and functional scores in 63% (5/8) when followed up for 60 d. More recently,

Table 3 Outcome scoring in both groups

Variables	Placebo (mean ± SD)			Botulinum toxin (mean ± SD)		
	Pre	Post	*P* value	Pre	Post	*P* value
VAS	6.7 ± 0.92	2.85 ± 1.08	< 0.001	6.25 ± 0.55	2.45 ± 0.99	< 0.001
ODI	34 ± 4.58	19 ± 4.29	0.001	33.5 ± 3.76	17 ± 3.72	0.007

VAS: Visual analog scale; ODI: Oswestry disability index.

Table 4 Change in the outcome scoring between the groups

Variables	Placebo (mean ± SD)	Botulinum toxin (mean ± SD)	*P* value
Mean change in VAS	3.85 ± 0.81	3.8 ± 0.89	0.427
Mean change in ODI	15 ± 1.50	16.5 ± 2.41	0.012

VAS: Visual analog scale; ODI: Oswestry disability index.

Sahoo *et al*[10] enrolled 19 patients with CLBP and injected them with 100 units of BoNT-A[10]. The authors found beneficial effects in them at two months, which persisted even at six months.

Foster *et al*[12] conducted the earliest double-blinded RCT study in the year 2001 with 31 patients of CLBP using 200 Ipsen units of BoNT-A[12]. At eight weeks, the BoNT-A group had more pain relief (9/15 *vs* 2/16, $P = 0.009$) and enhancement in the LPB functional scores (10/15 *vs* 3/16, $P = 0.011$). No patients experienced side effects. A year later, Subin *et al*[11] conducted a similar study wherein they compared nine patients of BoNT-A (100 Ipsen) to 10 patients of placebo and found pain reduction to be significant in the BoNT-A group (7/9 *vs* 0/10)[11]. About a decade later, Jazayeri *et al*[14] did a single-blinded RCT in 50 patients who again received 200 Ipsen units of BoNT-A[14]. The authors found that at 8 wk, patients had better pain relief (64% *vs* 12%, $P = 0.001$), and functional scores (68% *vs* 12%, $P = 0.005$) as compared to the saline group. Later, Machado *et al*[16] conducted a double-blinded RCT wherein 18 patients received a high dose (500-1000 units) of BoNT-A and 19 patients received normal saline[16]. The researchers only showed marginal improvement in the pain scores. Cogné *et al*[13] did a cross-over RCT wherein they proposed to have 60 patients; half of which were planned to receive 200 Ipsen units of BoNT-A, and another half were placebo with drugs change at 120 d[13]. The authors had to curtail their recruitment following no beneficial effect with a final size of 19. The authors concluded that there was no advantage of BoNT-A compared to placebo (30, 90, 120 d) regarding clinical outcome scores, quality of life, and spinal strength. Our study also showed no significant difference compared to the placebo regarding pain and functional scores similar to the above study. Clearly, the researchers have different dosages, with a range from 100-1000 units. The action is reversible, and therefore, the benefit has also been studied mostly in the short term (2 months), and this property has been used by Cogné *et al*[13] for drug crossover[13]. Only one study demonstrates benefits for up to 6 months[10].

No patient in our study had any adverse reaction. Jabbari *et al*[8] had 3 (4%) of patients, while Ney *et al*[21] had two patients with flu-like symptoms that resolved in 2-5 d[8,21]. Other researchers like Jazayeri *et al*[14] also did not report any complications[14].

Our study has a few limitations. CLBP is multifactorial, and this causal heterogeneity may affect the response to BoNT-A treatment. Better localization of the injected muscles with newer techniques, such as ultrasound/electromyogram, could help improve the results of BoNT-A. We have used a dose of 200 units, which can be considered inadequate in comparison to few studies that have utilized higher doses and found beneficial effects[8,16]. Nevertheless, future trials could be multicentric and conducted with larger doses to document any constructive effect. The strength of this study is it is an RCT. A negative finding of our study can help clinicians and researchers to consider against using a costly drug like BoNT-A for treating CLBP.

CONCLUSION

BoNT-A is found to have no advantage over the placebo in the short term for relieving pain and LBP scores in CLBP.

FOOTNOTES

Author contributions: Jain M, Tripathy SK and Mangaraj M conceived the idea; Jain M got the ethical clearance; Jain M, Varghese P, and Tripathy SK recruited the patients; Varghese P, Khan S, Jain M, and Tripathy SK followed up with them; Jain M, Khan S, and Mangaraj M prepared the initial draft of the manuscript, and the others (Tripathy SK and Varghese P) provided critical input; All authors read and

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Country of origin: India

ORCID number: Paulson Varghese 0000-0002-2323-4980; Sujit Kumar Tripathy 0000-0003-0179-9910; Manaswini Mangaraj 0000-0003-0179-9912.

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SYSTEMATIC REVIEWS

Retinoscopes: Past and present

Mutali Musa, Ehimare Enaholo, Babatunde Ismail Bale, Carlo Salati, Leopoldo Spadea, Marco Zeppieri

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Mutali Musa, Babatunde Ismail Bale, Department of Optometry, University of Benin, Benin 300283, Nigeria

Mutali Musa, Ehimare Enaholo, Department of Ophthalmology, Africa Eye Laser Centre, Benin 300105, Nigeria

Ehimare Enaholo, Department of Ophthalmology, Centre for Sight Africa, Nkpor 434101, Nigeria

Carlo Salati, Marco Zeppieri, Department of Ophthalmology, University Hospital of Udine, Udine 33100, Italy

Leopoldo Spadea, Eye Clinic, Policlinico Umberto I, "Sapienza" University of Rome, Rome 00142, Italy

Corresponding author: Marco Zeppieri, MD, PhD, Doctor, Department of Ophthalmology, University Hospital of Udine, p. le S. Maria della Misericordia 15, Udine 33100, Italy. markzeppieri@hotmail.com

Abstract

BACKGROUND

Retinoscopy is arguably the most important method in the eye clinic for diagnosing and managing refractive errors. Advantages of retinoscopy include its non-invasive nature, ability to assess patients of all ages, and usefulness in patients with limited cooperation or communication skills.

AIM

To discuss the history of retinoscopes and examine current literature on the subject.

METHODS

A search was conducted on the PubMed and with the reference citation analysis (https://www.referencecitationanalysis.com) database using the term "Retinoscopy," with a range restricted to the last 10 years (2013-2023). The search string algorithm was: "Retinoscopy" (MeSH Terms) OR "Retinoscopy" (All Fields) OR "Retinoscopes" (All Fields) AND [(All Fields) AND 2013: 2023 (pdat)].

RESULTS

This systematic review included a total of 286 records. Publications reviewed iterations of the retinoscope into autorefractors, infrared photo retinoscope, television retinoscopy, and the Wifi enabled digital retinoscope.

CONCLUSION
The retinoscope has evolved significantly since its discovery, with a significant improvement in its diagnostic capabilities. While it has advantages such as non-invasiveness and broad applicability, limitations exist, and the need for skilled interpretation remains. With ongoing research, including the integration of artificial intelligence, retinoscopy is expected to continue advancing and playing a vital role in eye care.

Key Words: Retinoscopy; Autorefractor; Refractive errors; Ophthalmology; Optics; Artificial intelligence

Core Tip: Retinoscopy is an important method used in the eye clinic for identifying and treating refractive problems. It has several benefits, such as being non-invasive, evaluating patients of all ages, and being helpful for individuals with poor cooperation or communication abilities. It is very helpful in the diagnosis of diseases like cataracts and amblyopia. New features have been added to retinoscopes as a result of technological advances. Contemporary retinoscopes come with digital screens, which make it simpler to analyze the findings. Others have combined the advantages of both with integrated autorefractor capabilities. Retinoscopes have evolved in the past decades to meet the current clinic needs.

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INTRODUCTION

Refractive errors are the leading cause of visual impairment worldwide[1]. Estimation of refractive errors can be carried out objectively or subjectively. Objectively, refraction is carried out by retinoscopy and auto-refractometry.

The retinoscope works on the principle of detecting the movement of a light beam reflected from the patient's retina. By analyzing the direction and speed of the reflected light, clinicians can determine the refractive error, such as myopia, hyperopia, or astigmatism. By their optical function, retinoscopes may also be improvised for the detection of multiple unestablished anterior segment pathologies, including aiding differential diagnosis of several subtypes of immature cataracts in low-resource clinical ophthalmic settings by observing differences in motions exhibited by lens opacities against the background red reflex. All illuminated portable devices can be used for this subjective assessment.

The retinoscope, particularly when used in cycloplegic conditions, proves to be a valuable tool for epidemiological purposes, aiding in screening for the distribution and development of refractive errors in infants and young children[2-4]. Furthermore, it acts as the benchmark for creating alternative tests or procedures aimed at enhancing the measurement or identification of clinically relevant refractive errors in the pediatric population[5]. Significant refractive errors contribute to avoidable vision impairment and amblyopia among pediatric age groups[6]. Regarding targeting community health efforts, the results of some studies suggest a greater predilection of amblyopia based on race as an independent factor; however, these inferences may have been influenced by geographical bias[7,8].

The optics of retinoscopy can be explained using Foucault's principle[9]. The retinoscope can also be used to measure leads and lags of accommodation at near using the Monocular Estimated Method of retinoscopy[10]. While it may not be employed frequently, the retinoscope has practical applications in clinical settings for measuring the amplitude of accommodation[11]. There are various tools for subjective refinement of astigmatic correction, including astigmatic fan dial, Jackson cross-cylinder[12], *etc*. Stenopaic slit refraction enables the refinement of moderate astigmatism in lower resource settings[13]. This paper sought to summarize the historical background and importance of this technique while highlighting the evolution of this procedure and the current advancements being made.

MATERIALS AND METHODS

A search was conducted on the PubMed and with the reference citation analysis (https://www.referencecitationanalysis.com) database using the term “Retinoscopy,” with a range restricted to the last 10 years (2013-2023). The search string algorithm was: "Retinoscopy" (MeSH Terms) OR "Retinoscopy" (All Fields) OR "Retinoscopes" (All Fields) AND [(All Fields) AND 2013: 2023 (pdat)]. Two of the authors scrutinized each publication record for relevance and a PRISMA [14] guideline was used to represent article discovery and is shown in Figure 1.

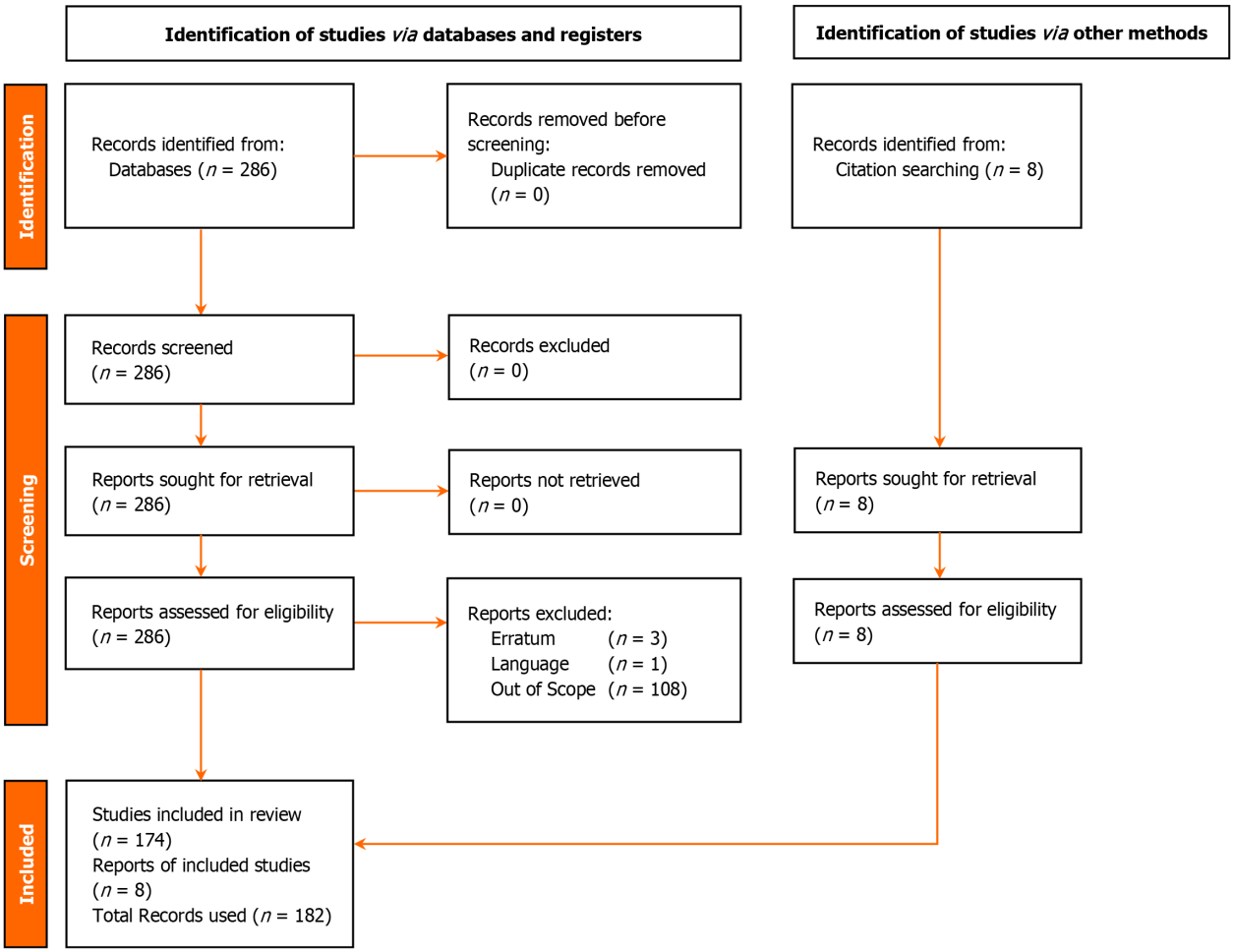

Figure 1 PRISMA guideline.

RESULTS

The search string returned a total of 286 records. Articles not in English language were excluded. Three records were excluded as they were erratum to other publications, and one was excluded as it was not in English. A total of 108 records were classified as out of scope and were therefore not included. The authors then searched the references and citations of the remaining 178 studies, further harvesting an additional eight studies reviewed in this paper.

DISCUSSION

Measurement of refractive errors

Refractive errors like hyperopia and myopia are lower-order aberrations generated *via* properties of the ocular refractive media in relation to globe anatomy[15]. The magnitude of either positive spherical defocus (with myopia)[16] or negative spherical defocus (with hyperopia) accounts for their visual significance[17]. "Astigmatism" is a second-order aberration contributed by differences between the eye's principal meridians[18]. Lower-order aberrations are corrected optically with spherical or spherocylindrical lenses. Clinical refraction is essential for deriving optimal corrections. Retinoscopy and the use of the auto-refractometer are good objective techniques for estimating magnitudes of spherocylindrical corrections. However, retinoscopy permits more procedural variability[19,20]. Novel self-contained darkroom refractive screeners have been shown to measure spherical equivalents similar to values determined from routine cycloplegic retinoscopy[21]; both tests reportedly varied only in magnitudes of spherical and cylindrical components[21]. The spherical-equivalent value represents an algebraic sum of the spherical component and the half of the cylinder in an optical prescription[22].

During retinoscopy and other reliable refraction procedures, the principal meridians are orthogonal in cases of regular astigmatism. The power meridian is steepest, while the axis meridian is flattest. When performing minus-cylinder refraction, neutralizing the power meridian *via* retinoscopy requires a less myopic/more hyperopic spherical component. An adequate cylindrical component then neutralizes the axis meridian[23]. On the other hand, the magnitude of a cylindrical component can be derived from the algebraic difference of both parts on an optical cross. Against-the-rule astigmatism exists with a minus cylinder axis along 90 degrees. Astigmatism is with-the-rule (WTR) when the minus

cylinder axis favors 180 degrees. WTR astigmatism is more tolerable[12] and common among younger demographic groups[24-27].

After retinoscopy, good subjective refraction is needed to account for objective over- or under-correction[28]. Amblyopia is a huge consequence of uncorrected or inadequately corrected refractive error[6]. Accurate determination of the magnitude and orientation of both manifest and cycloplegic astigmatism is essential to assuring good visual outcomes following keratorefractive surgery. When utilizing vector analysis in preparation for keratorefractive and refractive lens implant-based procedures, the preoperative best-correction is factored in determining optimal treatment parameters such as target-induced astigmatism and surgical-induced astigmatism (when retreatment protocols become necessary).

On-the-axis retinoscopy as a refractive technique is important in the examination, as well as the treatment of refractive errors and amblyopia[29]. Higher-degree refractive errors are more amblyogenic[30]. The maximum correction of significant refractive errors is essential in amblyopia prevention measures[31]; data acquired from amblyopia screening among preschoolers suggested that hyperopia > 2 D, astigmatism > 1 D, and anisometropia > 0.5 D were unilateral amblyogenic refractive factors[32]. On the other hand, bilateral amblyopia was most associated with bilateral hyperopia ≥ 3 D[32].

For very young child with significant refractive errors, refraction techniques can be optimized to aid favorable emmetropization[33,34]. In the setting of long-term optometric care, consistently greater-than-expected longitudinal increase in myopic spherical equivalents can aid in early diagnosis of progressive myopia, such that timely myopia control therapies can be instituted[35,36]. Among pediatric populations, myopia is more prevalent post-emmetropization [36], and can be worsened by dim light and near work[37]. Accordingly, hyperopia prevalence is inversely associated with pediatric age[38]. Preterm children may be an exception to this trend[39].

Modern autorefractors yield higher minus-powered spheres and lower plus-powered spheres/spherical equivalents [40]. To avoid over- or under-correction, good fogging techniques are a key step in carrying out subjective refraction. Binocular balancing techniques, although not the subjective refraction endpoint, can help stabilize the relative binocular accommodative stimulus. The Humphriss immediate contrast and prism-dissociated red-green balance methods enable better consistency of results[41].

Conducting refractive screenings for newborns allows for the early detection of refractive conditions' distribution, which could serve as risk factors for amblyopia[42] and other congenital ocular conditions like retinopathy of prematurity [43-45] and retinoblastoma[46]. For young children managed for retinopathy of prematurity, accurate cycloplegic retinoscopy post-treatment enables early detection and good long-term comparison of unwanted refractive consequences [47]. Pathologic changes in adults may also present with a shift in refractive values which may be picked up by retinoscopy[48].

Historical background and evolution of retinoscopy and autorefractors

Early retinoscopy systems were cumbersome, and they consisted of a wall-mounted illumination source: Initially a lamp or lit candle. The handheld unit consisted of a reflecting mirror which was then held perpendicular to the wall-mounted unit and the visual axis of the patient[23].

The irregular reflex seen in the eye when illuminated was first reported in 1859[49]. Cuignet had earlier characterized the changes in this reflex as the illuminating source changed in direction and location[49] but it was not until 1878 that Parent published the objective refraction technique[49]. Since then, retinoscopy has been the most reliable tool for determining objective refraction values. Schaeffel *et al*[50] developed the infrared retinoscope in 1987[50]. These earlier units enabled spot retinoscopy only. Over time, self-illuminated retinoscopes were developed, with the evolution of Copeland's streak retinoscope being a major landmark in the adoption of retinoscopy for broader modern-practice applications[23]. While the early models featured a simple mirror system, modern retinoscopes often employ complex optical designs, such as the streak retinoscope. Over time, retinoscopes have become more refined and user-friendly. These newer instruments offer improved accuracy and ease of use.

The recently introduced "Mirza" tele-lens retinoscopy emerges as a more precise and accurate refractive assessment method for evaluating refractive errors in young, uncooperative children and infants compared to the standard retinoscopy, proving effective in both non-cycloplegic and cycloplegic conditions[51].

Certain portable autorefraction devices are valuable substitutes for retinoscopy when screening and diagnosing refractive errors, particularly in low-income communities with constrained financial resources and a shortage of trained eye care professionals[52]. With tendencies similar to those observed during retinoscopy, non-cycloplegic and cycloplegic autorefraction yields distinct spherical equivalent values when employed for examining children and adolescents: Post-cycloplegic myopic readings often decrease in magnitude while post-cycloplegic hyperopic values increase in magnitude [53].

Retinoscopy-based screening tools have enabled epidemiological studies incorporating larger sample sizes of school-aged children[54]. The availability of more device options also offers variability for examining special-needs children; these advancements have also enabled the acquisition of more epidemiological data regarding vision problems among children with Down syndrome[55]. A streak retinoscope connected to a smartphone-based display system enabled trainer-trainee 'video-refractive retinoscopy' for easy description of retinoscope reflex properties in various refractive states and several other associated optical phenomena[56]. Other developments and the subsequent changes to advancement provided to the retinoscopic technique[50-62] are listed in Table 1.

Autorefractors began to come on the scene within the last 30 years[63]. An "auto-refracto-keratometer" denotes a unified device that combines the functionalities of an autorefractor and a keratometer, offering details on refractive error and corneal curvature[64]. Several autorefractometer devices have shown good levels of consistency[65]. Refraction outcomes from autorefractor models produced by reputable stalwart ophthalmic device companies have been comparable to those of dynamic retinoscopy[40].

Table 1 Evolution of retinoscopes presented in the literature

Year	Authors	Instrument	Advancement
1987	Schaeffel *et al* [50]	Infrared photo retinoscope	Ability to refract off-axis and peripheral areas of the cornea
1987	Miller *et al*[58]	Television retinoscopy	This setup used a live television camera to gather retinoscope reflex images for the purpose of teaching
2014	Chen *et al*[57]	Infrared retinoscopy	Extend the detection range of illuminating eccentricity making aberrations easier to detect
2014	Chan *et al*[60]	Digital retinoscope	Authors developed a digital retinoscope by connecting a smartphone to a streak retinoscope for training and demonstration
2019	Arnold *et al*[59]	School bus accommodation relaxing skiascopy	School bus accommodation relaxing skiascopy precisely estimates refractive errors including astigmatism in children without the need for cycloplegia
2022	Langue and Ajay[61]	Wifi enabled digital retinoscope	Authors attached a Wifi enabled camera to a retinoscope, allowing reflex to be viewed on other video terminals wirelessly for training
2022	Musch *et al*[62]	Welch Allyn spot vision screener model VS-100	This novel equipment detects refractive error binocularly. However, false negatives were noted

Autorefractors can be described as closed-field or open-field. The closed-field equipment has a target generated inside the autorefractor while in the open-field versions, the patient is encouraged to look through a clear opening[66]. Specialized camera-based equipment for screening amblyogenic factors such as ametropias, ocular deviations, and opacities like the medical technology and innovations photo screener and Fortune video refractor emerged at the turn of the century[67]. Screening devices such as the Retinomax autorefractor and SureSight Vision Screener showed good sensitivity for detecting significant refractive errors compared to non-cycloplegic retinoscopy[68].

The Plusoptix autorefractor emerged in 2004 and has been researched to show good agreement with cycloplegic retinoscopy[69,70]. Yet, additional research conducted by Saini *et al* reveals that in comparison to cycloplegic retinoscopy, the utilization of Plusoptix proves to be a more dependable method for determining the axis of the cylindrical component of refractive error in children[71]. The PlusoptiX photo screener has shown greater suitability for the detection of myopia [72]. However, both Pedia Vision and Plusoptix photo-screeners were found to overestimate the magnitude of myopia and astigmatism while also yielding underestimates of hyperopia[73].

Advantages and limitations of the retinoscope

The retinoscope is the most reliable tool for obtaining refractive values in children and individuals who cannot communicate optimally[74-83], and even newborns[84-90]. Retinoscopy is reported to be the most sensitive (78.6%) with a negative predictive value of 96.6%[91].

For assessing the spherical equivalent of subjective refraction in children, cycloplegic retinoscopy proves to be a superior method compared to autorefraction[92]. However, Akil *et al*[93] concluded that there is a strong correlation between cycloplegic retinoscopy and autorefraction values[93]. In situations where it is deemed necessary, non-cycloplegic retinoscopy proves beneficial for evaluating subjective refraction, particularly within school eye health programs[94-97]. In the pediatric ophthalmic examination, retinoscopy after cycloplegia is also more suitable for attaining optimal correction of hyperopia compared to other methods of objective refraction[98]. Among adults, retinoscopy also reduces the probability of hyperopic spherical equivalent under-correction compared to objective results from autorefractors[99]. Dynamic retinoscopy has also been used to determine near addition power in presbyopes[100].

Patients are required to fulfill less strict postural demands during retinoscopy compared to using common table-top autorefractors. Hence, retinoscopy is adaptable to the examination of those afflicted with significant musculoskeletal disorders, as well as children and adolescents presenting with signs of attention-deficit hyperactivity disorder and autism spectrum disorder (ASD), all of whom may have acquired abnormal head posture[101,102]. Special-needs children and young adolescents may also be hyper-reactive to closed-field autorefraction[102]. Young patients with less manifest ASD may also have suboptimal vergence/pseudo-vergence facility findings that may be missed when closed-field autorefraction is relied upon[103]. Evidence suggests that retinoscopes are useful tools for complementing several aspects of clinical research, or knowledge generation in the fields of vision science and translation to optometric practice[104,105].

In animal experiments aimed at studying refractive errors, retinoscopy is an accurate and rapid method of achieving this[106-119]. For this purpose, retinoscopy has been used to study spherical equivalent changes in guinea pigs to understand cellular mechanisms of axial length elongation, choroidal dynamics, and several specific exogenous associations[120-122]. In murine models, continuous retinoscopy under ametropic conditions has also been theorized with the construction of a skull-secured trial frame[123]. Retinoscopic values are also useful in intraocular lens calculations during equine cataract surgery[124].

Challenges of retinoscopy

The outbreak of coronavirus disease 2019 (COVID-19) and its resultant pandemic changed the practice policy of many clinics worldwide. Valuable in-person training hours were lost during the COVID-19 pandemic[125-127]. Thakur *et al* [128] also published a case series showing changes in the retinoscopy-based objective refraction endpoint after recovering from COVID-19[128]. Because the retinoscopic procedure requires that the clinician sit directly in front of patients and

make multiple contacts with lenses and equipment used by patients, several authors recommended discontinuing the procedure in favor of automated objective refractometry[129]. Photophobic patients may also become uncomfortable from the bright light of the retinoscope. Coulter *et al*[102], however, described using the Luneau Retinoscopy Rack and a video target at 10 feet to capture the attention of kids[102].

Gaining proficiency in performing retinoscopy portends slow learning curves, requiring a high volume of procedural repetitions[130]. Good clinical guidance and supervision of novice technicians by more experienced personnel serve to overcome challenges associated with the quality of patient care[131]. Also, the cooperation level of patients and the experience of the clinician can influence variations in retinoscopy findings[132]. Failure to attain optimal retinoscopic correction predisposes hyperopic school-aged children to accommodative and vergence anomalies[133].

Potential sources of error and factors affecting accuracy

Examiners' proficiency and experience are important factors influencing the accuracy of retinoscopy[134]. Bharadwaj *et al*[134] described a psychometric technique for predicting individual retinoscopists' accuracy of results[134]. Very high refractive errors can result in an atypically blurred 'starting-point' reflex, thus confusing the inexperienced examiner[23].

The choice of topical agents used for cycloplegic retinoscopy in young children and early adolescents can also result in variability, particularly for young hyperopic children[135]. Mydriatic agents such as tropicamide are listed in several works of literature as cycloplegics for pediatric ocular assessment; however, they exert weaker cycloplegic effects for young children (< 5 years) presenting with accommodative esotropia and high hyperopia[135]. To avert severe adverse events, it remains prudent to select concentrations of topical cycloplegic drops following due consideration of age, body weight, and pre-existing hypersensitivities for individual pediatric patients[136]. Marked pupil miosis associated with senescence, can make the retinoscopy reflex appear obscured, hence limiting accuracy[137,138].

It is, however, noted that retinoscopy is not the endpoint of the refractive process for the majority of patients and there are subjective steps to fine-tune the refractive prescription[139-141].

Diagnostic capabilities of retinoscopy

Generally, refraction is the mainstay for detecting the presence and magnitude of ametropias[142,143]. Refractive errors include hyperopia, myopia, and astigmatism. Retinoscopy is also especially useful in determining the presence and magnitude of astigmatism. Astigmatism may also be regular or irregular[144]. Pre- and cycloplegic retinoscopy findings are crucial in the differential diagnosis of near reflex spasm[145]. Objective refraction with cycloplegics is the standard of care among pediatric patients with significant visual anomalies[146]. Comparing non-cycloplegic and cycloplegic retinoscopy findings can help detect and diagnose accommodative dysfunctions[147]. Cycloplegia is attained by instilling drugs such as cyclopentolate, homatropine, and atropine to eliminate accommodation in the eye before refraction. Cycloplegia usually sets in about 30-40 min after the eyedrop has been instilled[148].

Cycloplegic/wet retinoscopy allows objective assessment of the eye's absolute refractive state[149]. Cyclopentolate, with its faster effect and shorter duration of recovery, is a better option for high-volume outpatient practices[150]. Research conducted by Groth *et al*[151] has suggested (albeit in a canine model) that cycloplegia may not produce statistically different results in retinoscopy[151]. Vasudevan *et al*[152] researched into differences in spherical endpoint between static retinoscopy in the dark as compared to cycloplegic retinoscopy. Their results show that there was no statistical difference between the two methods[152].

Mohindra near retinoscopy proves to be a beneficial technique for consistently screening the refractive status of children under 12 years old, providing reliable results comparable to those achieved with cycloplegic refraction[153].

Retinoscopy *via* the monocular estimation method is a subjective measure of the accommodative response (lead: With over-responsiveness, or lag: With under-responsiveness)[154]. The presence of a lead on accommodation seems to be a factor in myopic progression[155] and the development of amblyopia[156]. Another retinoscopic method applied in clinical settings is dynamic Nott retinoscopy, which assesses the precision of accommodation and proves beneficial for examining accommodative and binocular vision disorders at a point in time[157] or longitudinally[158]. The reduced accommodative facility at near and higher lag of accommodation was believed to predict myopia progression in adults [159]. In Nott's method, the patient wears his distance prescription and is asked to fixate on a target mounted on a calibrated ruler. The examiner observes the retinoscopic light reflex in the eye and adjusts his position forward and close to the patient or away from the eye until the refractive error is neutralized[160]. Notts retinoscopy is especially useful in screening for refractive errors in children with Down's syndrome[161]. Off-axis retinoscopy has gained some credence as a potential hypothetical measure of peripheral refraction[162].

The presence of a scissor-like reflex on the cornea during retinoscopy is one of the classical signs of keratoconus[163-165], a condition seen frequently among patients with long-standing vernal keratoconjunctivitis[166]. Yet, authors have suggested that this simple tool is not regularly used in the diagnosis of keratoconus[167]. In advanced keratoconus cases, a paracentral corneal oil-droplet sign and marked scissor reflex are confirmatory correlations with other non-retinoscopic clinical signs which include: Fleischer's ring, Munson and Rizzuti signs, Vogt's striae, and subepithelial apical scarring [168]. On the other hand, for earlier diagnosis of subclinical or 'Forme Fruste' keratoconus, patient groups with scissor retinoscopy reflexes, normal intraocular findings, and moderate-to-high astigmatism with corrected distance visual acuity < 6/9 require further assessment of central/apical corneal thickness, as well as biometry of the anterior and posterior corneal curvatures[168]. When available, Placido disc topography (for the air-tear-epithelial interface), scanning slit topography, and Scheimpflug imaging (capable of assessing posterior corneal elevation) are adjunctive to retinoscopy screening cues for true confirmation of preclinical/subclinical keratoconus[169]. This scissor-like retinoscopic reflex may serve as a useful lower-resource marker of irregular astigmatism[170].

In patients with unintended thicker flaps post-laser-assisted keratomileusis, a scissor motion seen on retinoscopy may be the first postoperative indication of wrong preoperative corneal biometry values[171]. In patients suspected of having a spasm of near reflex, a finding of > 2 diopters difference between standard and cycloplegic retinoscopy confirms the condition[172].

Current technology in objective refraction

Although cycloplegic retinoscopy is still considered the primary method for diagnosing refractive errors, challenges such as difficulty in obtaining cooperation from pediatric patients and the clinician's level of expertise have led to the emergence of modern technological alternatives, such as auto-refractometers[173]. Regarding autorefractors, while they are faster and demand less cooperation when used without cycloplegia, they produce more myopic outcomes that lack repeatability, especially within the pediatric population[174,175]. Likewise, photo-refractors, by accurately assessing refractive errors and amblyopic risk factors while overestimating myopia in children and hyperopia in adults, serve as a valuable and reliable alternative technology, particularly in communities with limited or no access to eye care services [176]. SureSight photo-refractors/Vision Screeners are advantageous for detecting hyperopia[177]. Photo-refractor technicians should, however, take individual and ethnic differences when calibrating for a refractive error measurement [178].

There is a possibility that automated devices, like the Plusoptix Power Refractor II, utilizing the eccentric photorefraction principle for detecting significant hyperopia in children, may lack the required level of accuracy in vision screening programs[179]. While the Plusoptix A09 photo screener serves as a beneficial tool for screening refractive errors in 5-to-15-year-old children, its effectiveness, particularly for myopic and astigmatic conditions, could be enhanced by combining it with retinoscopy[180]. Similar to other photorefraction technologies such as the Retinomax, Plusoptix, iScreen Vision Screener, and Adaptica 2WIN, the Spot Vision Screener captures and assesses images of the red reflex in the eyes to detect ametropia (primarily leaning towards myopia) in children starting from 6 mo old[181,182].

Compared to cycloplegic retinoscopy, the 2WIN-S photo-refractometer stands out as a highly dependable, swift, and portable device for evaluating refractive status in pediatric screening[183]. However, total reliance on the refraction measurements of screening tools can be unideal for making precise spectacle prescriptions[184]. The instaref R20, a portable/handheld auto refractometer manufactured based on the principles of Hartmann-Shack wavefront aberrometry, a wavefront sensor-based technology with high clinical usability over the years, showed good reliability and agreement with standard retinoscopy for use in pediatric evaluation[185]. A United States-based company created the Near Eye Tool for Refractive Assessment, a portable device attachable to smartphones, rapidly estimating refractive errors by displaying red-green line patterns through a pinhole optic aligned by the user[186,187]. Open-field autorefractors, such as the Shin-Nippon NVision-K 5001, provide a more dependable and precise assessment of refractive errors, specifically in children with hyperopia and oblique astigmatism, when compared to closed-field autorefractors like the Topcon KR-800[187].

Future technologies

The emerging Binocular Wavefront Optometer employs wavefront aberration principles and adaptive optics technology to efficiently and accurately assess children's refractive status, surpassing traditional autorefraction and retinoscopy under cycloplegic conditions with a superior 0.05 D-interval resolution compared to the standard 0.25 D-interval in optometry[188]. When compared to retinoscopy and autorefraction, the SVOne, a portable Hartmann-Shack wavefront aberrometer utilizing wavefront sensors and capable of connecting to a smartphone, objectively assesses the eye's refractive error[189].

QuickSee is an affordable and portable autorefractor utilizing wavefront aberrometry, capable of providing a satisfactory assessment of the eye's refractive status[190].

Utilizing wavefront aberrometry technology, the E-see autorefractor delivers a refractive error estimation that is more precise and consistent with retinoscopy compared to alternative autorefraction methods[191]. The SureSight Vision Screener, utilizing wavefront analytic technology, shows promise in evaluating the refractive status of children under three years old comparable to cycloplegic retinoscopy, albeit needing additional validation[192].

While the wavefront-based autorefraction measurements of children's refractive status in both non-cycloplegic and cycloplegic conditions show consistent astigmatic data, there exists a 0.5 D disparity in the spherical equivalent of the non-cycloplegic measurement, which can be mitigated through repeated measurements[193].

Geremias *et al*'s study revealed the Spot Vision Screener as an advanced and effective automated photo screening tool, proving superior in accurately measuring the refractive status of children below 3 years old (a risk factor of amblyopia) under cycloplegia conditions compared to retinoscopy, particularly beneficial in low-resource settings[194]. Likewise, additional research has affirmed the high reliability of the Spot Vision Screener in evaluating amblyopic risk factors among children with neurodevelopmental disabilities[195].

The Plusoptix S12-C photo screener proves to be a valuable and efficient tool for screening amblyogenic risk factors in children as young as 6 mo old, particularly in low-income communities[196]. Similarly, the Plusoptix A12-C photo screener is effective in detecting refractive amblyopia risk factors but not strabismic risk factors in children aged 3 to 4 years[197,198].

Artificial intelligence in retinoscopy

Integrating artificial intelligence (AI) into modern objective refraction techniques can be outlined to serve the following functions: Optimizing technical/operator training, and reducing patient/subject waiting time and discomfort[199-201].

The challenges with in-person examination created by the COVID-19 pandemic necessitated objective refraction simulations[126]. Chandrakanth *et al*[199] proposed a smartphone-based application for documenting retinoscopy called the Gimbalscope[199]. This device combines a smartphone with a traditional retinoscope and can be used as a teaching

tool for clinicians wanting to understand the reflex patterns seen during the procedure.

Researchers have experimented with integrating AI modalities with portable vision screeners. Handheld infrared eccentric automated refractors have also been implemented with advanced AI/deep learning algorithms which help minimize environmental and motion artifacts influencing their utility[200]. Similarly, pediatric vision screeners that measure perifoveal retinal birefringence have been optimized with artificial neural networks which detect central fixation and thus, obtain more accurate refraction measures in the setting of amblyopia and strabismus[201]. The development of predictive analytics for ocular refraction is an evolving research area in medical AI. The clinical significance of the Fusion Model-Based Deep Learning System (FMDLS), utilizing Retina Fundus Photographs, has been established in detecting spherical, cylindrical, and axis components of refractive errors, mirroring the effectiveness of cycloplegic refraction[202] while reducing human error. This particular retinal fundus photograph of the FMDLS correlated common features of the optic nerve head, fovea, and subretinal vascular reflectivity among myopes as predictors of the refractive error. As an improvement upon previous AI systems which yield output in spherical equivalent values, the FMDLS algorithm further highlighted optic disc orientation and macular area morphology as regions of interest in differentiating "WTR" from oblique forms of astigmatism; interracial variation was unaccounted for[202]. Training future advanced AI models of ocular refraction with datasets obtainable from wavefront sensor devices may help equate, or even surpass standard refractive measures acquirable with non-machine learning approaches.

CONCLUSION

In conclusion, the retinoscope has evolved significantly since its inception, adapting to changing technology and improving diagnostic capabilities. While it has advantages such as non-invasiveness and broad applicability, limitations exist and the need for skilled interpretation remains. Amblyopia is a main consequence of inappropriate refractive error correction during early childhood. Retinoscopy still represents a useful tool for ameliorating inadequate pediatric refractive error screening coverage in remote and underserved areas.

This study search was limited to retinoscopy. While this is a very common procedure, there is a paucity of related published data and a good number of studies returned by the search only mentioned it in passing while discussing entirely different topics. Widening the search criteria to include refraction could potentially have yielded more studies to review.

FOOTNOTES

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Country of origin: Italy

ORCID number: Mutali Musa 0000-0001-7486-8361; Ehimare Enaholo 0000-0002-2410-7510; Carlo Salati 0000-0003-4736-5296; Leopoldo Spadea 0000-0002-1190-3956; Marco Zeppieri 0000-0003-0999-5545.

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META-ANALYSIS

Anticoagulant use before COVID-19 diagnosis prevent COVID-19 associated acute venous thromboembolism or not: A systematic review and meta-analysis

Kinza Iqbal, Akshat Banga, Taha Bin Arif, Sawai Singh Rathore, Abhishek Bhurwal, Syeda Kisa Batool Naqvi, Muhammad Mehdi, Pankaj Kumar, Mitali Madhu Salklan, Ayman Iqbal, Jawad Ahmed, Nikhil Sharma, Amos Lal, Rahul Kashyap, Vikas Bansal, Juan Pablo Domecq

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Kinza Iqbal, Taha Bin Arif, Syeda Kisa Batool Naqvi, Muhammad Mehdi, Pankaj Kumar, Ayman Iqbal, Jawad Ahmed, Department of Internal Medicine, Dow Medical College, Karachi 74200, Pakistan

Akshat Banga, Department of Internal Medicine, Sawai Man Singh Medical College, Jaipur 302004, India

Sawai Singh Rathore, Department of Internal Medicine, Dr. Sampurnanand Medical College, Jodhpur 342003, Rajasthan, India

Abhishek Bhurwal, Department of Gastroenterology and Hepatology, Rutgers Robert Wood Johnson School of Medicine, New Brunswick, NJ 08901, United States

Mitali Madhu Salklan, Department of Internal Medicine, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak 124001, Haryana, India

Nikhil Sharma, Vikas Bansal, Juan Pablo Domecq, Department of Nephrology and Hypertension, Mayo Clinic, Rochester, MN 55905, United States

Amos Lal, Department of Medicine, Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN 55905, United States

Rahul Kashyap, Department of Research, Wellspan Health, York, PA 17403, United States

Co-first authors: Kinza Iqbal and Akshat Banga.

Co-corresponding authors: Vikas Bansal and Juan Pablo Domecq.

Corresponding author: Vikas Bansal, MBBS, Associate Research Scientist, Department of Nephrology and Hypertension, Mayo Clinic, 200 1st St SW, Rochester, MN 55905, Rochester, MN 55905, United States. bansal.vikas@mayo.edu

Abstract

BACKGROUND

Coagulopathy and thromboembolic events are associated with poor outcomes in coronavirus disease 2019 (COVID-19) patients. There is conflicting evidence on

the effects of chronic anticoagulation on mortality and severity of COVID-19 disease.

AIM

To summarize the body of evidence on the effects of pre-hospital anticoagulation on outcomes in COVID-19 patients.

METHODS

A Literature search was performed on LitCovid PubMed, WHO, and Scopus databases from inception (December 2019) till June 2023 for original studies reporting an association between prior use of anticoagulants and patient outcomes in adults with COVID-19. The primary outcome was the risk of thromboembolic events in COVID-19 patients taking anticoagulants. Secondary outcomes included COVID-19 disease severity, in terms of intensive care unit admission or invasive mechanical ventilation/intubation requirement in patients hospitalized with COVID-19 infection, and mortality. The random effects models were used to calculate crude and adjusted odds ratios (aORs) with 95% confidence intervals (95%CIs).

RESULTS

Forty-six observational studies met our inclusion criteria. The unadjusted analysis found no association between prior anticoagulation and thromboembolic event risk [n = 43851, 9 studies, odds ratio (OR)= 0.67 (0.22, 2.07); P = 0.49; I^2 = 95%]. The association between prior anticoagulation and disease severity was non-significant [n = 186782; 22 studies, OR = 1.08 (0.78, 1.49); P = 0.64; I^2 = 89%]. However, pre-hospital anticoagulation significantly increased all-cause mortality risk [n = 207292; 35 studies, OR = 1.72 (1.37, 2.17); P < 0.00001; I^2 = 93%]. Pooling adjusted estimates revealed a statistically non-significant association between pre-hospital anticoagulation and thromboembolic event risk [aOR = 0.87 (0.42, 1.80); P = 0.71], mortality [aOR = 0.94 (0.84, 1.05); P = 0.31], and disease severity [aOR = 0.96 (0.72, 1.26); P = 0.76].

CONCLUSION

Prehospital anticoagulation was not significantly associated with reduced risk of thromboembolic events, improved survival, and lower disease severity in COVID-19 patients.

Key Words: Prior anticoagulation; COVID-19; Prehospital anticoagulation; Chronic anticoagulation; Mortality; Severity; Thromboembolic events

Core Tip: Coronavirus disease 2019 (COVID-19) patients are at an increased risk of developing thromboembolic events and hypercoagulable disorders, which are poor prognostic indicators of COVID-19 disease. The results of different observational studies on the effects of chronic anticoagulation on the mortality and severity of COVID-19 disease in infected patients are inconsistent. This systematic review and meta-analysis provide a comprehensive assessment of the risk of thromboembolic events, mortality, and severity of COVID-19 disease in patients on prehospital anticoagulation treatment.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 and was later termed coronavirus disease 2019 (COVID-19)[1]. While Acute Respiratory Distress Syndrome (ARDS) was the predominant complication associated with a potentially fatal outcome, the infection progression could also be influenced by other risk factors like diabetes, coronary artery disease, chronic kidney disease, metabolic syndrome, psychiatric and neurological complications[2-12]. Due to the complexity of these complications, a standardized treatment approach for COVID-19 has yet to be universally agreed upon to guide healthcare professionals worldwide[10,13-15].

Studies have described that multi-organ involvement in COVID-19 is associated with endothelial dysfunction and thrombosis[16]. Moreover, COVID-19 has been observed to influence clotting system pathways and activate platelets, potentially resulting in vascular inflammation, hypercoagulability, and endothelial dysfunction[17]. As a result, thromboembolic events are found to be a common complication in these individuals, with an incidence rate of around 25% for Venous Thromboembolism (VTE)[16]. SARS-CoV-2-related coagulopathy is distinct from other causes of coagulopathy that result in VTE as it involves a significant element of inducing systemic inflammation and endothelitis, which might

not be effectively addressed by conventional anticoagulant treatment methods[18]. Regardless, emerging research evidence suggests that anticoagulation therapy has shown effectiveness in COVID-19 patients, particularly in noncritically ill hospitalized cases[19].

Studies mostly emphasize using anticoagulation therapy during hospitalization; however, the relationship between pre-existing chronic anticoagulation due to non-COVID-19 indication and clinical outcomes in COVID-19-infected patients remains largely unknown. This meta-analysis aims to analyze the relationship between VTE risk in COVID-19 disease and prehospital anticoagulation and to study the impact of prehospital anticoagulation on COVID-19 disease severity and mortality.

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were followed in this systematic review and meta-analysis[20]. The protocol for this meta-analysis was registered on PROSPERO (CRD42022-306893).

Data sources and literature search strategy

Two independent investigators (Iqbal K and Rathore SS) utilized databases, including LitCovid, WHO, and Scopus, to conduct a systemic literature search for relevant articles from December 2019 to June 30, 2023 (Supplementary Table 1). A combination of keywords, such as "anticoagulant", "direct oral anticoagulants", "DOAC", "vitamin K antagonist", "VKA", "dabigatran", "rivaroxaban", "apixaban", "edoxaban", "warfarin", "heparin", "prehospital", "prehospital", "preadmission", "pre-admission", "chronic", "long-term", "pre-hospital", and "prior", were searched electronically[21].

Study selection and inclusion criteria

Duplicates within the articles retrieved from our systemic search were identified and eliminated using Endnote (Clarivate Analytics, Thomson Reuters Corporation, Philadelphia, Pennsylvania). Two independent researchers (Iqbal K and Rathore SS) screened the articles based on their titles, abstracts, and keywords. The articles were subsequently assessed for relevance through full-text screening. Data review and collection were done by Bhurwal A, Iqbal A, Ahmed J, Iqbal K, Sharma N, Mehdi M, Kumar P, and Rathore SS. References to the shortlisted articles were also screened for additional studies. Any disagreement was resolved by discussing within the group or through an independent reviewer's (Bansal V) inputs. Articles that failed to fulfill the inclusion criteria were removed. For the meta-analysis, the inclusion criteria were: (1) All retrospective or prospective observational studies enrolling patients ≥ 18 years; (2) Studies that compared outcomes of COVID-19-infected patients who had been on anticoagulation before their COVID-19 diagnosis for any indication *vs* those that did not receive any prehospital anticoagulation; and (3) Studies including data (raw or adjusted) for at least one of the outcomes of interest. Exclusion criteria consisted of: (1) Studies without a control group; (2) Studies with no outcome of interest (new incidence of thromboembolic events, all-cause mortality, severity of COVID-19 (defined as per WHO Ordinal scale 6-9); and (3) Literature reviews or narrative reviews or case reports.

Our primary outcome of interest was the incidence of VTE in COVID-19-infected adults on chronic anticoagulation prior to the infection. Mortality and disease severity were considered secondary outcomes. COVID-19 disease severity was based on the WHO ordinal scale[22]. A WHO clinical progression scale score of 6-9 indicated severe COVID-19, often necessitating intensive care unit (ICU) admission. In cases where this scale was not used, the highest WHO ordinal scale event was used as a substitute for severe infection. If ICU admission rates were unavailable, intubation or non-invasive mechanical ventilation rates were considered to assess COVID-19 severity. The cases included SARS-CoV-2 positive hospitalized patients with prehospital use of anticoagulants. The controls were COVID-19-positive hospitalized patients with no history of prehospital anticoagulation. Both raw data and their adjusted estimates were derived for the meta-analysis. Estimates from studies that performed propensity-matched scoring were also considered as adjusted estimates.

Statistical analysis

We used Review Manager v.5.4 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2014)[23] for unadjusted and adjusted analysis and Comprehensive Meta-Analysis software package 154 (Biostat, Englewood, NJ, United States)[24] for the meta-regression analysis. For the unadjusted analysis, we calculated the Mantel Haenszel crude odds ratios (ORs) using a random-effects model with a 95% confidence interval (95%CI). We also calculated the adjusted OR (aOR) with 95%CI to measure adjusted estimates. Adjusted estimates were used for the primary analysis to produce more reliable results as it considers the baseline differences between the two groups (prior anticoagulant users and non-users) that may influence the outcomes. In all cases, significance was defined by a P-value < 0.05, and all analyses utilized a random-effects model. Statistical heterogeneity was gauged among the included articles using the Higgins I^2 statistics, and I^2 > 50% was regarded as substantial heterogeneity. To reduce the inherent selection bias in observational studies, a subgroup analysis based on study design was performed[25].

We conducted univariate and multivariate meta-regression analyses with random effects (maximum likelihood approach) to examine potential study variations that could impact the effect magnitude. The age and gender distribution of the study sample, the study's country of origin, and the percentage of participants with comorbid conditions like obesity, diabetes, pulmonary illness, dyslipidemia, cardiovascular disease, and hypertension were all considered potential sources of variability. If covariates significantly (P < 0.05) altered the relationship between mortality or severity in COVID-19 hospitalized patients and prehospital anticoagulant usage, they were chosen for additional modeling. Two models were developed: one for mortality and the other for the severity of the condition. Then, using P = 0.05 as the

cutoff threshold for elimination, preselected factors were included in a manual backward and stepwise multiple meta-regression analysis. $P < 0.05$ ($P < 0.10$ for heterogeneity) was used to define statistical significance. The meta-analysis and meta-regression tests were all two-tailed.

Risk of Bias and Quality assessment

Two investigators (Kumar P, Iqbal A) assessed the included studies' methodological quality through the Newcastle-Ottawa Scale (Supplementary Table 2)[26]. The following categories were used to evaluate every study: (1) Study group selection; (2) Study group comparability; and (3) Determination of the desired outcomes (Supplementary Table 2). The publishing bias likelihood was tested using Egger regression methods, and the results were visually examined using funnel plots (Supplementary Figure 1). The degree of evidence certainty at the outcome level was evaluated using the GRADE pro profiler (GRADE working group, McMaster University, and Evidence Prime Inc; Supplementary Table 3)[27].

RESULTS

The initial database search produced 2056 articles of potential relevance. After eliminating the duplicates, 956 articles were filtered for appropriateness and relevancy using their titles, abstracts, and keywords. Of them, 86 full-text papers relevant to the manuscript's objectives were examined. The final analysis included 46 studies comprising 41 cohort studies (36 retrospective, 2 ambispective, and 3 prospective), three case-control studies, and one cross-sectional study. The PRISMA flowchart outlining the search process is shown in Figure 1. Table 1 provides an overview of the study characteristics of the included publications, and Table 2 shows the studies reporting adjusted estimates along with the covariates for which the estimates are adjusted.

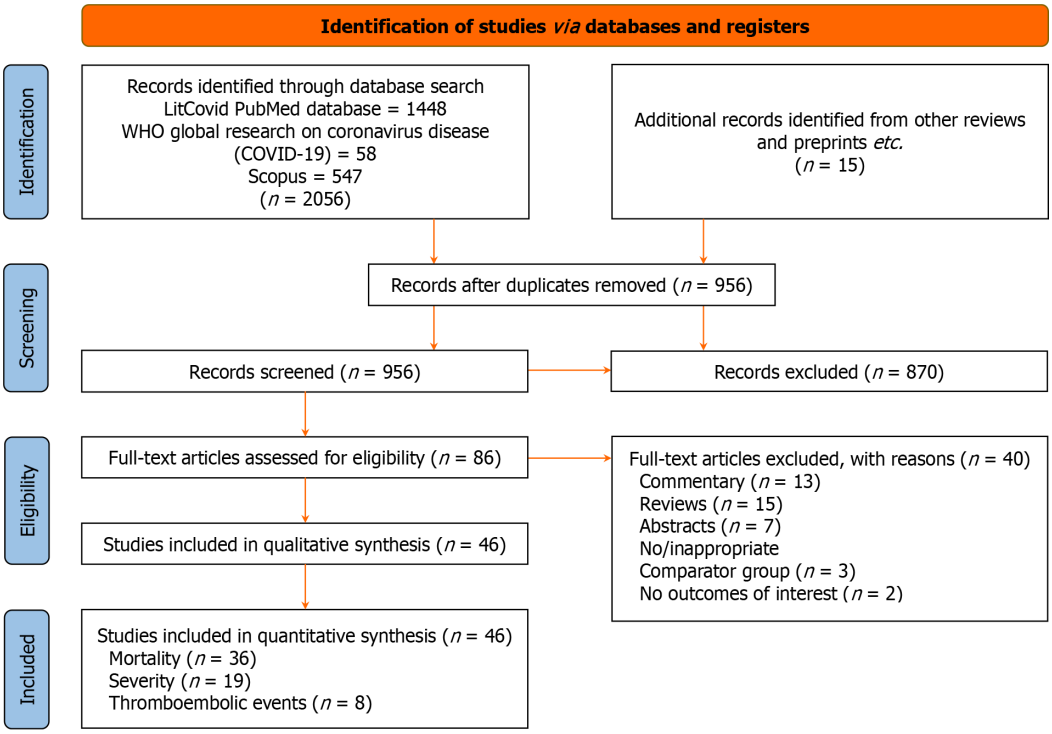

Figure 1 PRISMA flow diagram.

Out of the 46 studies included in our meta-analysis, 26 studies[28-54] described using both direct oral anticoagulants (DOACs) and vitamin K antagonists (VKAs). Five studies[55-59] had only DOAC as the prehospital anticoagulant, while a single study by Ménager *et al*[60] included patients only on VKAs. The anticoagulant type was unspecified in the remaining 13 studies[36,61-72]. In the 32 studies[28-35,37,39-51,53-60,73,74] studying DOACs Apixaban, Dabigatran, Edoxaban, and Rivaroxaban were the most commonly used, while on the other hand, VKAs were represented mostly by Warfarin. However, in two studies[30,60], Acenocoumarin was also taken into consideration. Supplemental prehospital use of other anticoagulants, including LMWH, Fondaparinux, and Enoxaparin, was observed in five studies[30,35,47,49,51].

Quality assessment and publication bias

The included studies' methodological quality assessment demonstrated that 21 studies[25,28,30,34,36,38,39,43,44,50,54,56,

Table 1 Study characteristic table for included studies

Ref.	Country of study	Study design	Setting	Total COVID-19 positive patients	Total patients with pre-admission anticoagulation	Type of anticoagulant	Duration of anticoagulant	Indication for anticoagulant Use	Definition Of Severity	Mean age ± SD (yr)	Female sex proportion (%)	Diabetes proportion (%)	Hypertension proportion (%)	Pulmonary disease proportion (%)	Arrhythmia proportion (%)
Ageno *et al* [28], 2021	Italy	Retrospective observational study	Inpatient hospitalized	4396	43	DOAC or VKA	-	AF	NA	-	-	-	-	-	56.4
Arachchillage *et al*[29], 2022	United Kingdom	Ambispective cohort study	Inpatient hospitalized	5883	963	DOAC (rivaroxaban, apixaban, edoxaban, dabigatran, or VKA (warfarin)	-	VTE or heart disease or AF	ICU admission	-	44.81	28.96	47.12	24.55	-
Aslan *et al*[55], 2021	Turkey	Retrospective cohort study	Inpatient hospitalized	1710	79	DOAC (dabigatran, rivaroxaban, apixaban, edoxaban)	-	AF, venous thrombosis	ICU admission	62 (52-71)	50.5	27	42	6	5
Bauer *et al*[71], 2021	United States	Case–control study	Hospitalized and non-hospitalized patients	1449	-	-		-	Admitted to the hospital/died	54.7 ± 22.5	63	17	36	22	-
Boari *et al*[68], 2020	Italy	Retrospective cohort study	Inpatient hospitalized	258	29	-	-	-	NA	71.0 ± 13.8	32.9	26	58.5	14	-
Brouns *et al* [53], 2020	Netherlands	Retrospective case-series	Inpatient hospitalized	101	18	DOAC or VKA	-	-	-	-	-	-	-	-	-
Buenen *et al* [40], 2021	Netherlands	Cohort study	Hospitalized and non-hospitalized patients	497	110	DOAC or VKA	-	-	NA	-	36.2	20.5	52.1	26	-
Chocron *et al* [41], 2021	France	Retrospective cohort study	Inpatient hospitalized	2878	382	DOAC or VKA	-	AF, VTE	ICU admission	-	40.3	30.3	75.3	-	24.8
Corrochano *et al*[30], 2022	Spain	Retrospective observational study	Inpatient hospitalized	1598	155	VKA (warfarin or acenocoumarol), DOAC (dabigatran, rivaroxaban, apixaban or edoxaban), or	-	-	ICU admission	66.5 (17.1)	47.1	20	50.8	-	-

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Covino *et al* [42], 2021	Italy	Retrospective observational study	Emergency department	184	92	DOAC or VKA	1 month	AF	NA	84 (81-87)	50	18.5	41.8	17.4	-
Denas *et al* [43], 2021	Italy	Retrospective observational study	Hospitalized and non-hospitalized patients	4697	651	VKA, NOAC (dabigatran, rivaroxaban, apixaban) or edoxaban	6 months	AF	ICU admission	-	45.6	24.1	87.3	-	-
Fauvel *et al* [54], 2020	France	Retrospective observational study	Inpatient hospitalized	1240	136	VKA 47 (3.8), NOAC 78 (6.3), heparin 11 (0.9)	-	-	ICU admission and mechanical ventilation	-	-	-	-	-	-
Flam *et al*[59], 2021	Sweden	Prospective cohort study	Inpatient hospitalized	459402	103703	DOAC (dabigatran, apixaban, rivaroxaban or edoxaban)	0–6 months [7394 (7.1%)]; 7–24 months [29012 (28.0%)]; > 24 months [67245 (64.8%)]	AF and atrial flutter	Hospital admission and ICU admission	-	-	-	-	-	-
Fröhlich *et al* [31], 2021	Germany	Retrospective cohort study	Inpatient hospitalized	6637	731	DOAC or VKA	6 months	AF	NA	-	50	25	93	-	76
Fumagalli *et al* [44], 2022	Italy	Retrospective cohort study	Inpatient hospitalized	176	91	DOAC or VKA	-	AF	NA	-	48.3	33	71	21	11.9
Gülcü *et al*[32], 2022	Turkey	Retrospective cohort study	Inpatient hospitalized	5575	451	DOAC (rivaroxaban, apixaban, edoxaban, dabigatran), and VKA (warfarin)	-	-	NA	64 (51–74)	49.8	26.9	49.5	13.9	5.2
Hanif *et al*[33], 2020	United States	Retrospective cohort study	Inpatient hospitalized	58	33	DOAC (rivaroxaban, apixaban, edoxaban, and dabigatran), VKA (warfarin)	-	-	NA	-	34.5	-	-	-	-
Harrison *et al* [34], 2021	United States	Retrospective cohort study	Inpatient hospitalized	1027	132	DOAC (rivaroxaban, apixaban, dabigatran), and VKA (warfarin)	-	-	NA	-	52.5	38.7	74.3	-	21.1

Ho *et al*[45], 2021	United States	Retrospective cohort study	Hospitalized and non-hospitalized patients	28076	304	VKA (warfarin) or DOAC (dabigatran)	Within 3 months prior to SARS-Cov-2 diagnosis	-	ICU admission	-	51.6	8.3	7.2	-	-
Hozayen *et al* [35], 2021	United States	Prospective cohort study	Outpatient and inpatient	5597	160	Enoxaparin, VKA (warfarin), DOAC	Within 3 months prior to SARS-Cov-2 diagnosis	-	NA	51 ± 22	57.1	15.7	34.7	4.5	12
Iaccarino *et al* [72], 2021	Italy	Cross-sectional study	Inpatient hospitalized	2377	125	DOAC	6 months	AF, mechanic valvularreplacement, pulmonary thromboembolism prophylaxis	ICU admission	68.2 ± 0.38	37.3	18	59	-	4.7
Klok *et al*[61], 2020	Denmark	Retrospective cohort study	Intensive care	184	17	-	-	-	NA	-	-	-	-	-	-
Li *et al*[36], 2020	China	Ambispective cohort study	Inpatient hospitalized	547	16	-	-	-	Severe COVID-19 infection	60 (48-69)	49.1	15.1	30.3	3.1	-
Lodigiani *et al* [73], 2020	Italy	Retrospective cohort study	Inpatient hospitalized	388	33	DOAC or VKA	-	-	ICU admission	66 (55–75)	32	22.7	47.2	9	-
Ménager *et al* [60], 2020	France	Retrospective cohort study	Inpatient hospitalized	82	9	VKA (warfarin or acenocoumarol or fluindione)	-	-	NA	88 (85–92)	47.6	23.2	63.4	-	36.6
Middeldorp *et al*[62], 2020	Netherlands	Retrospective cohort study	Inpatient hospitalized	198	19	-	-	AF	ICU admission	61± 14	34	-	-	-	-
Natali *et al* [63], 2020	United States	Retrospective case control study	Inpatient hospitalized	400	22	-	-	-	NA	-	-	-	-	--	-
Olcott *et al*[46], 2021	England.	Retrospective cohort study	Inpatient hospitalized	309	81	DOAC or VKA	-	-	NA	-	47.9	-	-	-	-
Parker *et al* [47], 2021	United Kingdom	Retrospective cohort study	Inpatient hospitalized	1032	164	DOAC, VKA, LMWH, fondaparinux	Taking the anticoagulant for > 1 month	AF, VTE, metallic heart valve, LV thrombus	ICU admission	71 (56–83)	44.9	29.3	44.3	-	-
Philipose *et al* [66], 2020	United Kingdom	Retrospective cohort study	Inpatient hospitalized	466	68	-	-	-	NA	-	-	-	50.2	28.1	-
Reilev *et al* [64], 2020	Denmark	Cohort study	Community-managed and hospitalized	11122	577	-	At least one filled prescription within 6 months prior to the test date	-	ICU admission	48 (33–62)	62	6.4	24	12	4.6

Rieder *et al* [37], 2022	Multi-Country	Retrospective cohort study	Hospitalized and outpatient	1433	334	VKA or non-VKA DOAC (rivaroxaban, apixaban, edoxaban, dabigatran etexilate)	-	AF	NA	-	39.8	31.2	86.5	-	25.4
Rivera-Caravaca *et al* [58], 2021	International, HOPE COVID-19 Registry	Retrospective cohort study	Inpatient hospitalized	1002	110	DOAC or VKA	-	AF, VTE, mechanical heart valves	NA	-	40.8	31.2	82.1	18.3	-
Rivera-Caravaca *et al* [38], 2021	United States, Trinetx	Cohort study	Hospitalized and outpatient	26006	13003	DOAC (dabigatran, apixaban, rivaroxaban or edoxaban)	1 yr	-	NA	-	48.4	37.9	71.9	18.3	48.1
Rodríguez-Molinero *et al* [67], 2020	Spain	Retrospective cohort study	Inpatient hospitalized	418	34	-	-	-	Need for oxygen therapy through a nonrebreather mask or mechanical ventilation	65.4 ± 16.6	43.1	23.7	52	9.8	10.8
Rossi *et al*[57], 2020	Italy	Retrospective observational study	Outpatient	70	26	DOAC (eivaroxaban, apixaban, edoxaban, dabigatran)	Regularly taken by the patient for at least 6 months	AF, pulmonary embolism, or DVT	NA	-	50	25.7	61.4	15.7	-
Russo *et al*[48], 2022	Italy	Retrospective observational study	Inpatient hospitalized	467	87	DOAC (edoxaban, dabigatran, rivaroxaban, apixaban), VKA (warafrin)	-	AF, prosthetic heart valve, venous thromboembolism	NA	-	33.3	25.3	74	18.8	-
Ruzhentsova *et al*[56], 2021	Italy	Retrospective cohort study	Outpatient	76	26	DOAC (rivaroxaban, apixaban, dabigatran)	-	-	NA	-	56.6	26.3	77.6	-	36.8
Schiavone *et al* [74], 2021	Italy	Retrospective cohort study	Inpatient hospitalized	844	65	DOAC or VKA	-	-	ICU admission	63.4 ± 16.1	38.3	16.6	45.1	7.4	9.2
Sivaloganathan *et al*[49], 2020	United Kingdom	Case-control study	Inpatient hospitalized	180	31	VKA (warfarin), DOAC (dabigatran, rivaroxaban, apixaban), or	-	-	ICU admission	-	-	-	-	-	-

						LMWH									
Spiegelenberg *et al*[50], 2021	Netherlands	Retrospective cohort study	Inpatient hospitalized	1154	190	DOAC or VKA	-	AF, VTE, mechanical valve replacement, cardiac arrest in history, or unknown (5%)	ICU admission	-	32.2	27.3	53.8	26.1	-
Tehrani *et al* [69], 2021	Sweden	Retrospective cohort study	Inpatient hospitalized	255	49	-	-	-	NA	66 ± 17	41	31	54	13	-
Togano *et al* [39], 2021	Japan	Retrospective cohort study	Inpatient hospitalized	4026	105	VKA (warfarin), DOAC (dabigatran, rivaroxaban, apixaban, or edoxaban)	-	-	Mechanical ventilation/ supplemental oxygen/SPO_2 ≤94% on room air/tachypnea	52.0 (34–69)	40.1	14.1	19	8.1	-
Tremblay *et al* [70], 2020	United States	Retrospective cohort study	Hospitalized and ambulatory patients	3772	241	-	-	-	Intubation-mechanical ventilation	56.6 (18.2)	45.2	-	-	14.9	-
van Haaps *et al* [51], 2021	Denmark	Retrospective cohort study	Inpatient hospitalized	3006	445	DOAC, VKA, LMWH	-	-	ICU admission	-	35.1	37.8	59.5	8.3	-
Wargny *et al* [65], 2021	France	Retrospective cohort study	Inpatient hospitalized	2796	501	-	-	-	NA	69.7 ± 13.2	36.3	100	76.8	9.6	-

Defined according to the 2019 clinical practice guideline from the Infectious Diseases Society of America and the American Thoracic Society for diagnosis and treatment of adults with community acquired pneumonia. DOAC: Direct-acting oral anticoagulants; VKA: Vitamin K antagonists; ARDS: Acute respiratory distress syndrome; NOAC: Non-Vitamin K oral anticoagulants; AF: Atrial fibrillation; VTE: Venous thromboembolism; DVT: Deep venous thrombosis; CAD: Coronary artery disease; NA: Not applicable; IQR: Interquartile range; OAC: Oral anticoagulant.

58-60,63,66,68,69,71,72,73] had a high quality with very low risk of bias, while 24 studies[29,31-33,35,37,40-42,46-49,51,55,57,62,64,65,67,70,73,74] were of moderate quality (Supplementary Table 2). Out of the 24 studies with moderate quality, 11 studies[29,33,35,37,41,46-49,52,62] had an unclear risk of bias (not enough information to make a clear judgment), and the remaining 13 studies[31,32,40,46,55,57,64,65,67,70,73,74] had a low risk of bias, but some other potential flaws. Therefore, every study was approved for inclusion in the quantitative analysis. A single study was of poor quality and potentially high risk of bias[53]. Supplementary Figure 1 displays the publishing bias funnel plots. The results showed no discernible publication bias, and Supplementary Figure 1 displays the various *P*-values from Egger's regression test. We also evaluated the certainty of evidence at the outcome level using GRADE pro profiler (GRADE working group, McMaster University, and Evidence Prime Inc; Supplementary Table 3)[20].

Primary outcome

Thromboembolic event rate: A total of nine studies[29,30,37,40,45,50,54,60,70] evaluated the unadjusted risk, while six studies[29,45,50,54,58,61]calculated the adjusted risk between prehospital anticoagulation and new thromboembolic events in COVID-19 infected patients. Three studies[45,50,62] defined thromboembolic events as VTE, while three others

Table 2 Studies reporting adjusted estimates and the factors for which they are adjusted

Ref.	Outcome	Adjusted estimates
Ageno *et al*[28], 2021	Mortality	Age, gender, and heparin use after admission, history of acute MI, T2D, HTN, cancer, COPD, renal function and CRP at hospital entry
Arachchillage *et al*[29], 2022	90-day mortality, thrombosis, and ICU admission	Age, gender, BMI, antiplatelet treatment prior to admission, autoimmune disease, malignancy, hypercholesterolaemia, heart disease, T2D, smoking status, liver disease, lung disease, existing renal failure and whether renal failure was dialysis dependent
Aslan *et al*[55], 2021	In-hospital mortality	Age, male gender, T2D, ferritin, d-dimer, neutrophil, lymphocyte, creatinine, CRP, SaO_2, procalcitonin, DOAC, HTN, HF, AF, CAD, COPD, systolic blood pressure and hematocrit in univariable logistic regression analysis
Buenen *et al*[40], 2021	All-cause mortality within 30 d	Age, sex, symptom duration, home medication, and comorbidities
Covino *et al*[42], 2021	All-cause in-hospital death.	Age, sex, comorbidity (categorized as CCI < 3 or CCI ≥ 3), and illness severity at admission (categorized as NEWS < 6 or NEWS ≥ 6)
Gülcü et al[32], 2022	In-hospital all-cause mortality	Age, gender, HTN, DM, HF, CAD, eGFR, albumin, CRP, D-dimer, hemoglobin, platelet count, LDH, and oxygen saturation variables
Bauer *et al*[71], 2020	Severity	Age and gender
Ménager *et al*[60], 2020	7-day mortality	Age, sex, severe undernutrition, T2D, HTN, prior MI, HF, prior stroke and/or TIA, CHA_2DS_2-VASc score, HAS-BLED score, and eGFR
Philipose *et al*[66], 2020	Mortality	Age and gender
Rieder *et al*[37], 2022	COVID-19 related mortality	Age, gender, BMI and smoking status, the phase of disease at diagnosis, solid tumor, AF, CAD, prior MI, peripheral artery disease, HTN, cerebrovascular disease, and T2D
Rivera-Caravaca *et al*[38], 2021	Mortality and the composite of any thrombotic or thromboembolic event	Age, gender and ethnicity, all the included comorbidities
Rodríguez-Molinero *et al*[67], 2020	Mortality	Age, sex, obesity, and corticosteroids
Russo *et al*[48], 2022	Mortality	Age, arterial HTN, T2D, CAD, HF, previous stroke
Schiavone *et al*[74], 2021	Mortality	Age > 65 years, male gender, CAD, CKD, COPD, HF, OAC, PaO2/FiO2, hydroxy-chloroquine, tocilizumab, antivirals, heparin
Spiegelenberg *et al*[50], 2021	All-cause in hospital mortality and ICU admission	Age, sex, body mass index, active malignancy, COPD, T2D, HTN, CAD, MI, HF, non-ischaemic cardiomyopathy, previous heart surgery, electronic heart device, cerebrovascular accident, peripheral artery disease, immunosuppressive medication, no ICU policy
Togano *et al*[39], 2021	Severity (mechanical ventilation/ supplemental oxygen/SpO_2 ≤ 94% on room air/tachypnea)	Age, gender, BMI, smoking, alcohol consumption, myocardial infarction/congestive heart failure, peripheral artery disease, cerebrovascular disease, dementia, paralysis, COPD, liver dysfunction, hypertension, hyperlip-idemia, diabetes, obesity, leukemia, lymphoma, immunosuppression
Tremblay *et al*[70], 2020	All-cause mortality, mechanical ventilation	Age, gender, race, CCI and obesity
van Haaps *et al*[51], 2021	21-day all-cause mortality and ICU admission	Age, gender, T2D, HTN, CKD, asthma, obesity, time in pandemic, center, chronic cardiac disease, malignancy, liver disease, dementia, organ transplant, autoimmune disorder, and rheumatic disorder
Wargny *et al*[65], 2021	Death within 28 d	Age
Harrison *et al*[34], 2021	21-day all-cause in hospital mortality	Age, gender, and confounding variables
Iaccarino *et al*[72], 2021	Mortality, ICU admission	Age, multimorbidity (combined in the CCI score), and gender
Hozayen *et al*[35], 2021	Mortality	Age, sex, self-identified race/ethnicity (as a proxy for social, not biological risk factors), Elixhauser comorbidity score, and the presence/absence of any cardiovascular, immunological or hematological comorbidities
Ho *et al*[45], 2021	ICU admission, VTE, and mortality between date of SARS-CoV-2 diagnosis and 45 d after diagnosis	Age, sex, race/ethnicity, body mass index, CCI, HTN, T2D, and smoking history as well as the week of SARS-CoV-2 diagnosis
Denas *et al*[43], 2021	ICU admission and all-cause mortality	Age, sex, HF, HTN, cancer, T2D, history of stroke/TIA, previous bleeding, history of MI, peripheral artery disease, abnormal renal function, abnormal hepatic function, use of antiplatelet drugs, NSAIDs and statin use
Corrochano *et al*[30], 2022	All-cause mortality and ICU admission	Sex, age, CCI, and antithrombotic therapy

Chocron *et al*[41], 2021	In-hospital mortality and ICU admission	Sex, age, cardiovascular comorbidities (history of HTN, dyslipidemia, BMI, T2D, and current smoking), plasma creatinine level (μmol/L), CRP (mg/L), fraction of inspired oxygen, the degree of pulmonary lesions with ground-glass opacities and areas of consolidation, and the use of in-hospital anticoagulation (preventive low or high dose and therapeutic dose)

T2D: Type 2 diabetes mellitus; HTN: Hypertension; HF: Heart failure; CAD: Coronary artery disease; MI: Myocardial infarction; AF: Atrial fibrillation; TIA: Transient ischemic attack; CKD: Chronic kidney disease; COPD: Chronic obstructive pulmonary disease; VTE: Venous thromboembolism; OAC: Oral anticoagulant; DOAC: Direct oral anticoagulant; CRP: C-reactive protein; LDH: Lactate dehydrogenase; BMI: Body mass index; CCI: Charlson comorbidity index; NEWS: National Early Warning Score; ICU: Intensive care unit; NSAIDs: Non-steroidal anti-inflammatory drugs; eGFR: Estimated glomerular filtration rate; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; COVID-19: Coronavirus disease 2019.

[29,30,37] included both arterial and venous thromboembolic events, and the remaining three[40,70] did not clarify their definition of thromboembolic events.

Overall, anticoagulation prior to COVID-19 diagnosis suggested a reduction in the unadjusted risk of new VTE in adult patients in these nine studies but did not attain statistical significance [n = 43851, OR = 0.67 (0.22, 2.07); P = 0.49; I^2 = 95%; Figure 2A]. Similarly, in the predefined subgroup analysis, the use of VKA[40,50,54] [2658 participants, OR = 0.32 (0.05, 1.98); P = 0.22; I^2 = 63%; Supplementary Figure 2A] or DOAC[40,50,54] [2699 participants, OR = 0.36 (0.10, 1.25); P = 0.11; I^2 = 51%; Supplementary Figure 2B] or any anticoagulants [40960 participants, OR = 1.03 (0.26, 4.08); P = 0.97; I^2 = 96%; Supplementary Figure 2C] did not show a statistical significant reduction of new VTE event risk associated with COVID-19 infection (P value for subgroup differences = 0.70, I^2 = 0%). Our prespecified adjusted meta-analysis on six studies[29,45,50,54,61,74] did not achieve statistical significance in the lower odds of thromboembolic events in patients suffering from COVID-19 [aOR = 0.87 (0.42, 1.80); P = 0.71; I^2 = 99%; Figure 3A].

Secondary outcomes

Mortality: A total of 35[28-35,37,38,40,41,43,44,46-48,50,51,53,55-57,59,60,63-70,72,74] studies reported mortality in prior anticoagulant users (2388 deaths out of 110975 patients; 2.1%) *vs* non-users (9457 deaths out of 96317 patients; 9.8%). Prior anticoagulation was found to significantly increase the risk of mortality in COVID-19 patients in the unadjusted analysis [OR = 1.72 (1.37, 2.17); P < 0.00001; I^2 = 93%; Figure 2B). Subgroup analysis by the type of anticoagulation medication showed that prior VKA use [19747 participants, OR = 1.91 (1.20, 3.06); P = 0.007; I^2 = 83%; Supplementary Figure 3A] and any anticoagulant use [43643 participants, OR = 1.88 (1.40, 2.52); P < 0.00001; I^2 = 94%; Supplementary Figure 3B] was associated with an increased mortality risk. However, prior use of DOAC [22374 participants, OR = 1.42 (0.95, 2.12); P = 0.08; I^2 = 87%; Supplementary Figure 3C] and the adjusted estimates of 28 studies[28,30,32,34,35,37,38,40-45,48,50,51,53,55,57,60,61,65-67,70,72,74] [aOR = 0.94 (0.84, 1.05); P = 0.31; I^2 = 65%; Figure 3B] revealed no statistically significant association between prehospital anticoagulation and mortality in patients hospitalized for COVID-19.

COVID-19 disease severity: Overall, 22 studies[30,31,33,36,39,41,43,47-51,55,56,59,62,64,67,70,72,73,74] documented the association between prehospital anticoagulation and COVID-19 infection severity. ICU admission was the surrogate marker of COVID-19 severity in 15 studies, while mechanical ventilation was used in five studies, and one study used ARDS development during hospitalization. Six hundred seventy-one patients out of 103,703 who received anticoagulants before COVID-19 diagnosis developed severe COVID-19 infection, while 6155 out of 78890 non-users progressed to severe COVID-19 illness. We derived no statistically significant association between prehospital anticoagulation and COVID-19 disease severity [OR = 1.08 (0.78, 1.49); P = 0.64; I^2 = 89%; Figure 2C]. On carrying out a subgroup analysis based on the type of anticoagulants, VKAs [6947 participants, OR = 1.26 (0.57,2.77); P = 0.57; I^2 = 82%; Supplementary Figure 4A], DOACs [149564 participants, OR = 1.12 (0.58, 2.15); P = 0.74; I^2 = 88%; Supplementary Figure 4B], and any anticoagulant use [36854 participants, OR = 1.07 (0.72, 1.58); P = 0.75; I^2 = 89%; Supplementary Figure 4C] all reported non-significant association with severe COVID-19 disease. Similarly, prehospital anticoagulation was not significantly associated with COVID-19 disease severity in the adjusted analysis [12 studies[29,30,39,41,43,45,50,51,59,70-72]; aOR = 0.96 (0.72, 1.26); P = 0.76; I^2 = 80%; Figure 3C].

Multivariate meta-regression model for mortality outcome

To account for variations in the correlation between mortality and prehospital anticoagulant use, a multivariate meta-regression was performed. The findings demonstrated that when considered collectively, the proportion of age, diabetes, female gender, hypertension, and pulmonary diseases was significant. These variables accounted for R^2 = 90% of the difference in mortality between studies (Figure 4A).

Multivariate meta-regression model for severity outcome

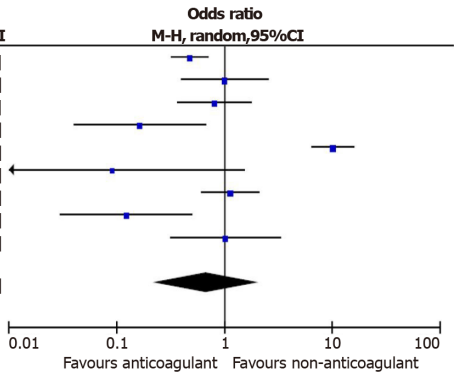
Multivariate meta-regression was employed to take into consideration variations in the correlation between COVID-19 severity and prehospital anticoagulation. Together, age, female gender, and the proportion of hypertension, pulmonary disease, and diabetes were found to be significant covariates. Figure 4B demonstrates that these factors, all together, explained R^2 = 100% for heterogeneity in severity among the included studies.

A

Study or subgroup	Anticoagulant users Events	Total	Non-anticoagulant users Events	Total	Weight	Odds ratio M-H, random,95%CI
Arachchillage *et al*. 2021	28	963	292	4920	12.5%	0.47 [0.32, 0.70]
Buenen *et al*. 2021	6	110	21	387	11.7%	1.01 [0.40, 2.56]
Corrochano *et al*. 2021	7	155	80	1443	11.9%	0.81 [0.37, 1.78]
Fauvel *et al*. 2020	2	125	101	1115	10.6%	0.16 [0.04, 0.67]
Ho *et al*. 2021	22	304	212	27772	12.4%	10.14 [6.44, 15.98]
Middeldorp *et al*. 2020	0	19	39	179	7.0%	0.09 [0.01, 1.54]
Rieder *et al*. 2021	14	334	41	1099	12.2%	1.13 [0.61, 2.10]
Spiegelenberg *et al*. 2021	2	190	77	964	10.6%	0.12 [0.03, 0.50]
Tremblay *et al*. 2020	3	241	43	3531	11.1%	1.02 [0.31, 3.32]
Total (95%CI)		**2441**		**41410**	**100.0%**	**0.67 [0.22, 2.07]**
Total events	84		906			

Heterogeneity: Tau^2 = 2.58; Chi^2 = 150.13, df = 8 (P < 0.00001); I^2 = 95%

Test for overall effect: Z = 0.69 (P = 0.49)

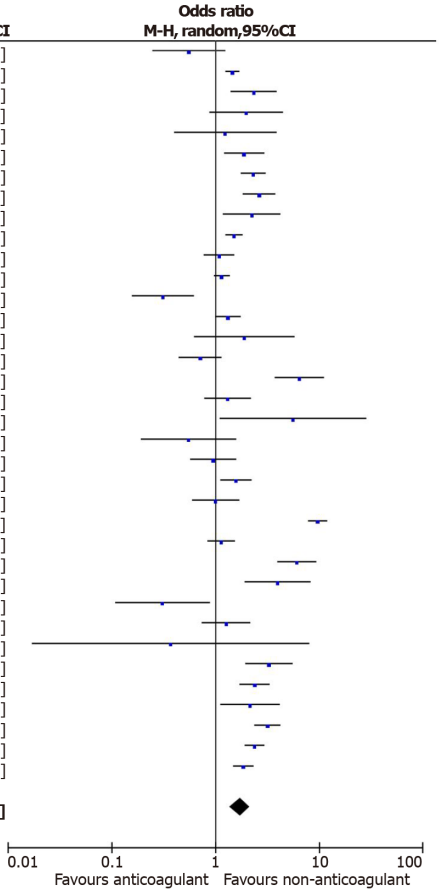


B

Study or subgroup	Anticoagulant users Events	Total	Non-anticoagulant users Events	Total	Weight	Odds ratio M-H, random,95%CI
Ageno *et al*. 2021	10	43	39	110	2.4%	0.55 [0.25, 1.24]
Arachchillage *et al*. 2021	346	963	1373	4920	3.4%	1.45 [1.25, 1.68]
Aslan *et al*. 2021	23	79	244	1631	2.9%	2.33 [1.41, 3.87]
Boari *et al*.2020	11	29	54	229	2.4%	1.98 [0.88, 4.45]
Brouns *et al*.2020	9	16	26	51	1.9%	1.24 [0.40, 3.83]
Buenen *et al*.2021	46	110	107	387	3.0%	1.88 [1.21, 2.92]
Chocron *et al*.2021	84	382	268	2466	3.3%	2.31 [1.76, 3.04]
Corrochano *et al*.2021	55	155	249	1443	3.2%	2.64 [1.85, 3.77]
Covino *et al*. 2021	38	92	22	92	2.7%	2.24 [1.19, 4.22]
Denas *et al*. 2021	189	651	866	4046	3.3%	1.50 [1.25, 1.81]
Flam *et al*.2020	140	103703	46	36875	3.2%	1.08 [0.78, 1.51]
Fröhlich *et al*. 2021	233	731	1400	4824	3.4%	1.14 [0.97, 1.35]
Fumagalli *et al*. 2021	21	91	33	67	2.6%	0.31 [0.16, 0.61]
Gülcü *et al*. 2021	62	451	554	5124	3.2%	1.31 [0.99, 1.74]
Hanif *et al*.2020	14	33	7	25	1.9%	1.89 [0.62, 5.77]
Harrison *et al*.2021	24	132	213	894	3.0%	0.71 [0.44, 1.13]
Hozayen *et al*. 2021	17	160	99	5437	2.9%	6.41 [3.73, 11.01]
Iaccarino *et al*.2021	18	125	257	2252	2.9%	1.31 [0.78, 2.19]
Ménager *et al*. 2020	3	9	6	73	1.3%	5.58 [1.11, 28.16]
Natali *et al*. 2020	5	22	52	149	2.0%	0.55 [0.19, 1.57]
Olcott *et al*. 2021	38	81	110	228	2.9%	0.95 [0.57, 1.58]
Parker *et al*. 2021	66	164	260	868	3.2%	1.57 [1.12, 2.22]
Philipose *et al*.2020	29	68	170	398	2.9%	1.00 [0.59, 1.68]
Reilev *et al*.2020	163	577	414	10545	3.3%	9.63 [7.84, 11.84]
Rieder *et al*. 2021	76	334	227	1099	3.2%	1.13 [0.84, 1.52]
Rivera-Caravaca *et al*. 2020	75	110	233	892	3.1%	6.06 [3.95, 9.30]
Rodri´guez-Molinero *et al*.2020	15	34	64	384	2.5%	3.95 [1.91, 8.18]
Rossi *et al*.2020	7	26	24	44	2.0%	0.31 [0.11, 0.88]
Russo *et al*.2021	23	87	84	380	2.9%	1.27 [0.74, 2.16]
Ruzhentsova *et al*.2021	0	26	2	50	0.5%	0.37 [0.02, 7.91]
Schiavone *et al*.2021	29	65	154	779	2.9%	3.27 [1.94, 5.50]
Spiegelenberg *et al*. 2021	75	190	207	964	3.2%	2.39 [1.72, 3.31]
Tehrani *et al*.2020	20	49	50	206	2.7%	2.15 [1.12, 4.13]
Tremblay *et al*.2020	81	241	486	3531	3.2%	3.17 [2.39, 4.21]
van Haaps *et al*. 2021	194	445	629	2561	3.3%	2.37 [1.93, 2.92]
Wargny *et al*. 2021	149	501	428	2293	3.3%	1.84 [1.48, 2.29]
Total (95%CI)		**110975**		**96317**	**100.0%**	**1.72 [1.37,2.17]**
Total events	2388		9457			

Heterogeneity: Tau^2 = 0.41; Chi^2 = 501.36, df = 35 (P < 0.00001); I^2 = 93%

Test for overall effect: Z = 4.62 (P < 0.00001)



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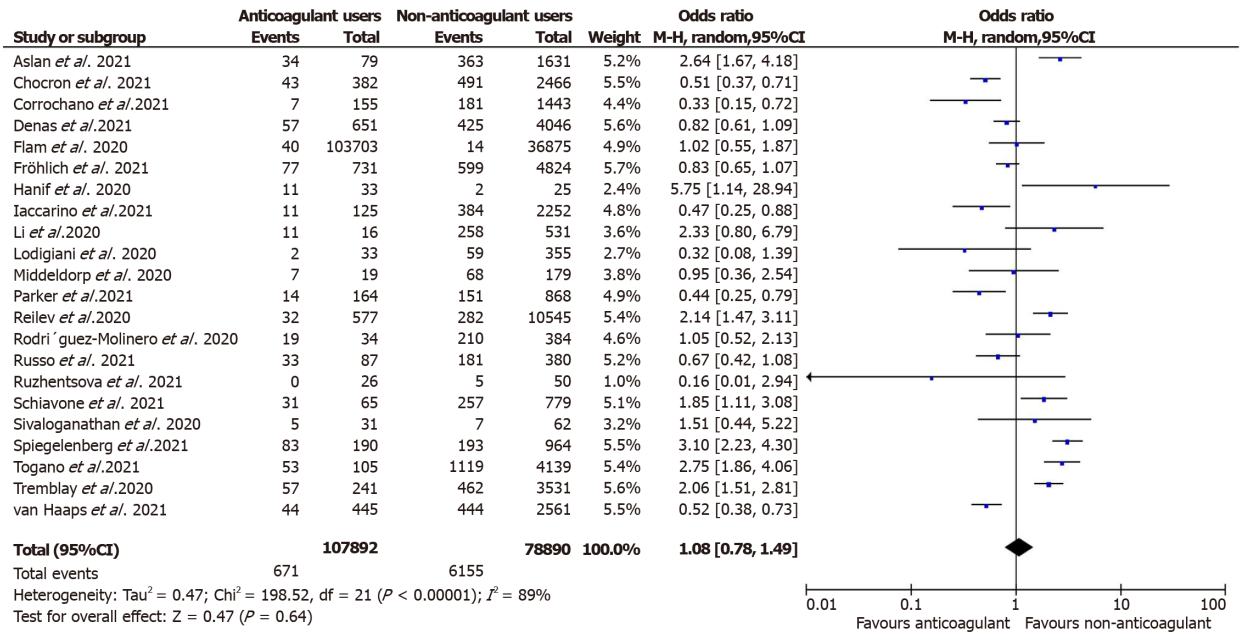


Figure 2 Unadjusted meta-analysis for mortality, severity thromboembolic events in prehospital use of anticoagulants *vs* control cohort in COVID-19. A: Unadjusted thromboembolic events in prehospital use of anticoagulants *vs* control cohort; B: Unadjusted mortality in prehospital use of anticoagulants *vs* control cohort; C: Unadjusted severity in prehospital use of anticoagulants *vs* control cohort.

DISCUSSION

Our analysis offered a thorough examination of the risk of thromboembolic events, mortality, and severity of COVID-19 disease when anticoagulant medication was used prior to hospitalization. The meta-analysis identified no significant association between prehospital anticoagulation and decreased thromboembolic events risk and reduced COVID-19 disease severity. Though the unadjusted analysis exhibited a rise in mortality risk of COVID-19 patients with prior anticoagulation, adjusting the estimates revealed no significant difference in the odds of thromboembolic events, mortality, and severity between COVID-19 infected patients on prehospital anticoagulation and those without antecedent anticoagulation treatment.

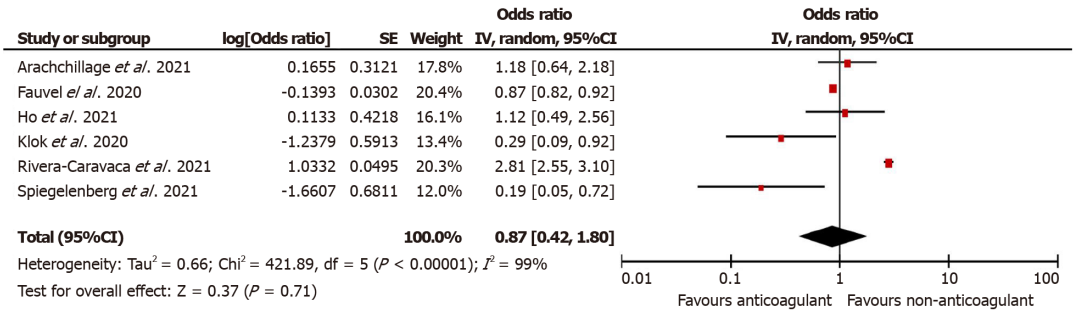
Literature review and analysis of existing research

VTE: Coagulopathy and thromboembolic events are described as independent poor prognostic indicators in COVID-19 [58,75]. Hospitalized COVID-19 patients tend to have a higher incidence of VTE than individuals with other illnesses[76], yet the exact mechanism of hypercoagulability remains unclear. COVID-19 infection can induce hyperinflammation[77], leading to endothelial dysfunction[78-80], platelet activation, blood stasis[58], and microvascular inflammation[81], all of which can influence consequent respiratory distress and other organ dysfunctioning[78-80]. This acute inflammatory state also increases the arterial and venous TE risk[61].

While ample evidence supports anticoagulation benefits during hospitalization[82-84], the role of prehospital anticoagulation before COVID-19 diagnosis, particularly in thromboembolic event incidence, lacks sufficient data. Despite anticoagulation, people on long-term oral anticoagulation therapy that started prior to COVID-19 infection may be more prone to thrombosis because of the existing SARS-CoV-2 infection[45]. Initial research suggests potential negative effects of VKAs on COVID-19[50]. SARS-CoV-2 infected individuals have reduced extra-hepatic vitamin K stores, which could be further decreased with VKAs[50]. However, studies have produced conflicting results regarding prehospital anticoagulation's impact on COVID-19 patients' thromboembolic risk, with some studies showing increased thromboembolic event risk[58]. In contrast, others observed benefits from prehospital anticoagulation in reducing COVID-related TE risk [29,50]. However, as shown in both unadjusted and adjusted analyses, our study did not find a significant reduction in thromboembolic events among COVID-19 patients receiving prehospital anticoagulation.

Mortality: Diverse study outcomes exist on chronic anticoagulation's impact on COVID-19 mortality. Tremblay *et al*[70] noted higher mortality among prehospital anticoagulated COVID-19 patients, yet non-significance emerged after age, sex, race, obesity, and Charlson Comorbidity Index adjustment. Rivera-Caravaca *et al*[58] reported higher all-cause mortality rates in prehospital DOAC-treated patients, remaining significant post-adjustment. Conversely, studies like Fumagillin *et al*'s[44] observed lower mortality rates in pre-COVID-19 anticoagulated patients[41,43,44,68]. Our research aligns with studies[41-51,69] and an earlier meta-analysis published by Kamel *et al*[85], revealing no substantial prehospital anticoagulation-associated mortality impact. Unlike Kamel *et al*[85], our study comprehensively explores pre-admission anticoagulation effects on VTE risk, severity of disease, and mortality in COVID-19.

A



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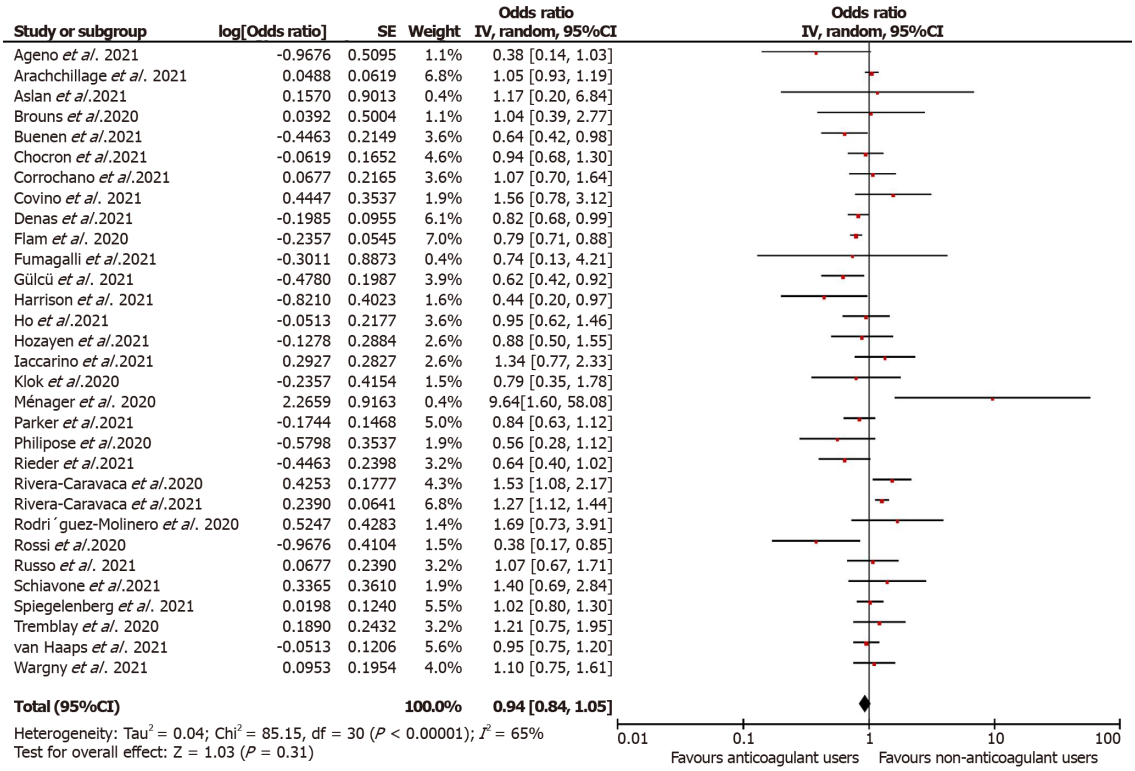

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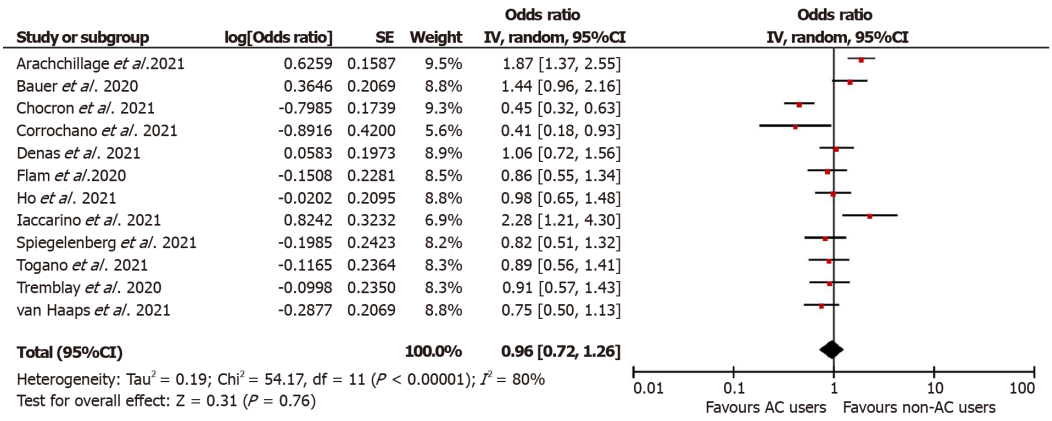
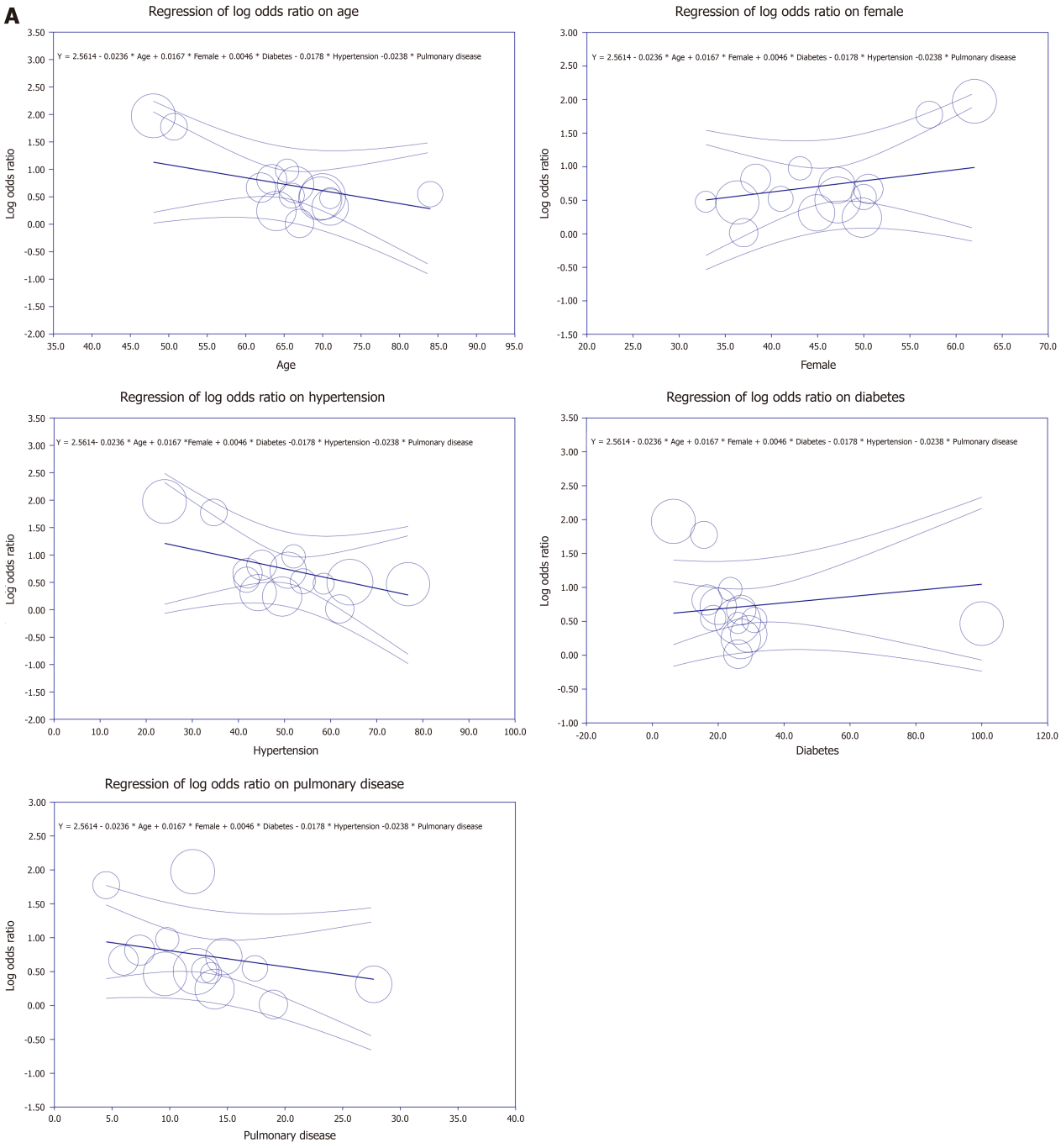


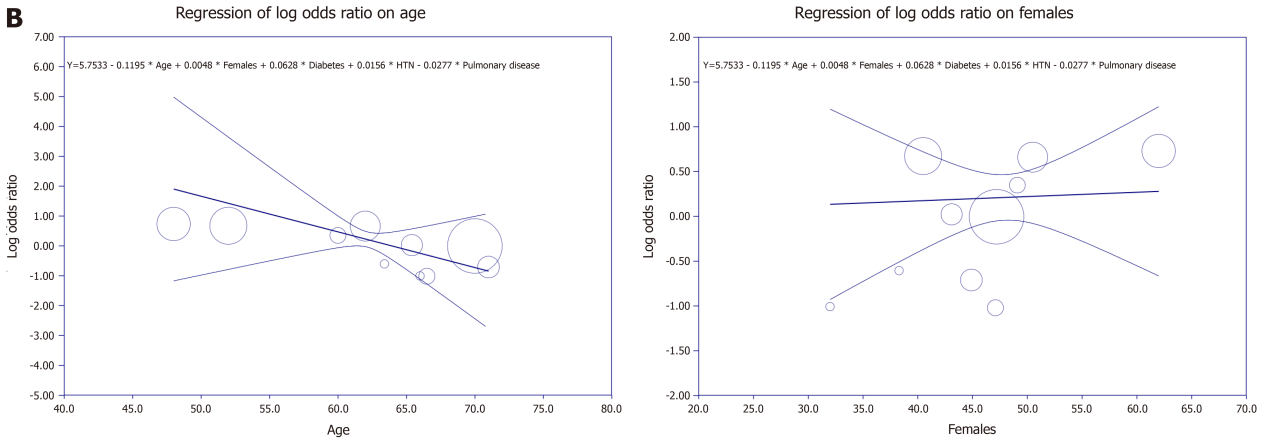
Figure 3 Adjusted meta-analysis for mortality, severity thromboembolic events in prehospital use of anticoagulants *vs* control cohort in COVID-19. A: Adjusted thromboembolic events in prehospital use of anticoagulants *vs* control cohort; B: Adjusted mortality in prehospital use of Anticoagulants *vs* control cohort; C: Adjusted severity in prehospital use of Anticoagulants *vs* control cohort.

Severity: Divergent results have been observed regarding the effect of anticoagulation on the severity of COVID-19[47,48,52,64,66,74]. Aslan *et al*[55] revealed significantly higher ICU admission, ventilation, oxygen therapy, and mortality rates with DOAC use. Yet, Corrochano *et al*'s[30] study found ICU admission reduction with prehospital anticoagulation after age, sex, and CCI adjustment. Similarly, Iaccarino *et al*[72] linked oral anticoagulants to ICU admission risk reduction. However, their cross-sectional design for hypothesis generation might influence outcomes[33].

A


Regression of log odds ratio on age
Y = 2.5614 - 0.0236 * Age + 0.0167 * Female + 0.0046 * Diabetes - 0.0178 * Hypertension -0.0238 * Pulmonary disease
Log odds ratio
Age
Regression of log odds ratio on female
Y = 2.5614 - 0.0236 * Age + 0.0167 * Female + 0.0046 * Diabetes - 0.0178 * Hypertension -0.0238 * Pulmonary disease
Log odds ratio
Female
Regression of log odds ratio on hypertension
Y = 2.5614- 0.0236 * Age + 0.0167 *Female + 0.0046 * Diabetes -0.0178 * Hypertension -0.0238 * Pulmonary disease
Log odds ratio
Hypertension
Regression of log odds ratio on diabetes
Y = 2.5614 - 0.0236 * Age + 0.0167 * Female + 0.0046 * Diabetes - 0.0178 * Hypertension - 0.0238 * Pulmonary disease
Log odds ratio
Diabetes
Regression of log odds ratio on pulmonary disease
Y = 2.5614 - 0.0236 * Age + 0.0167 * Female + 0.0046 * Diabetes - 0.0178 * Hypertension -0.0238 * Pulmonary disease
Log odds ratio
Pulmonary disease

B


Regression of log odds ratio on age
Y=5.7533 - 0.1195 * Age + 0.0048 * Females + 0.0628 * Diabetes + 0.0156 * HTN - 0.0277 * Pulmonary disease
Log odds ratio
Age
Regression of log odds ratio on females
Y=5.7533 - 0.1195 * Age + 0.0048 * Females + 0.0628 * Diabetes + 0.0156 * HTN - 0.0277 * Pulmonary disease
Log odds ratio
Females

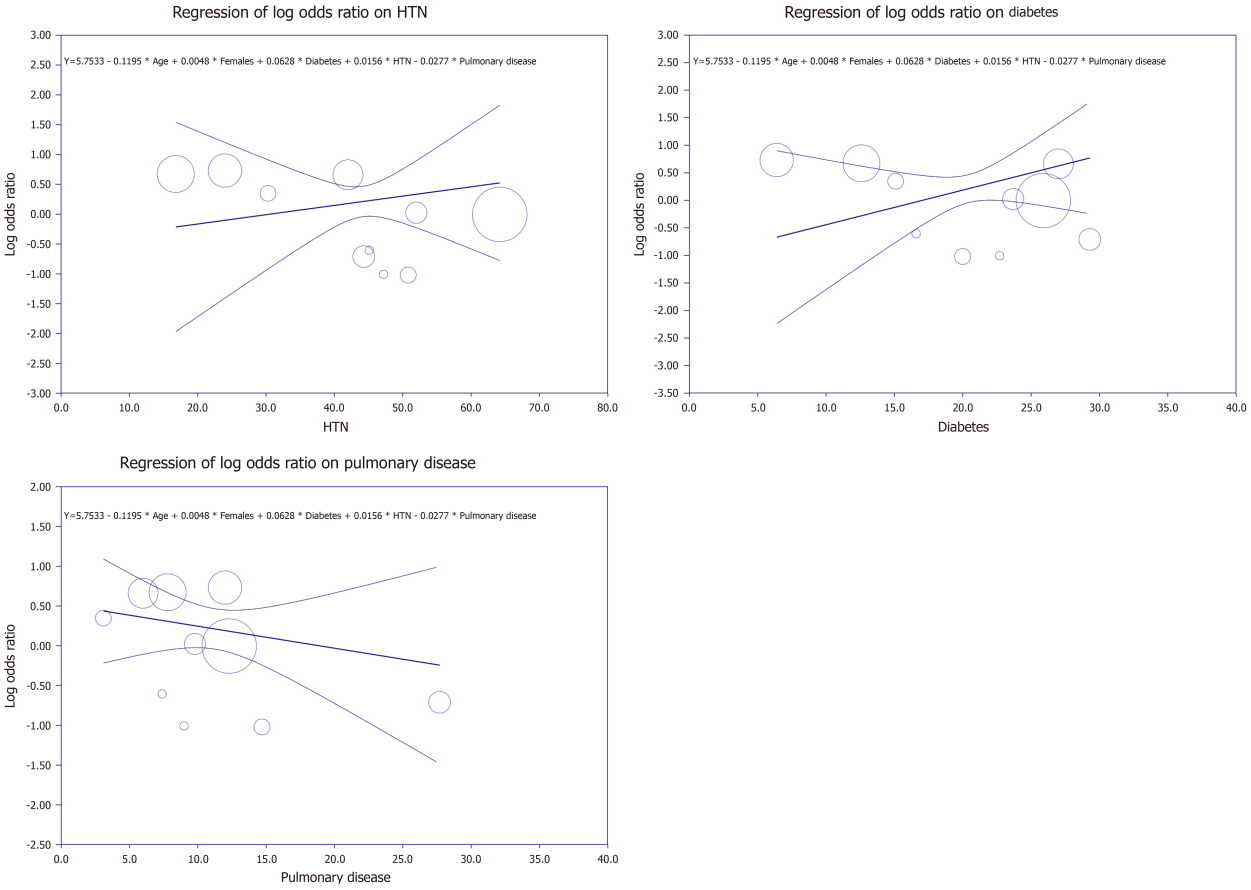


Figure 4 Meta-regression analyses. A: Mortality meta-regression analysis; B: Severity meta-regression analysis.

Assessment of potential biases

The existence of selection bias and confounding bias is one possible explanation for inconsistent results among studies[29,30,49,55,58], as some studies include patients with cardiovascular or coagulation issues (poorer premorbid state). Certain studies neglect the effects of other medications during hospitalization[84]. Another reason can be the nature of the study, as observational and retrospective studies limit the causal inference[27-30,55], even though propensity score matching efforts[58], as propensity scoring is dependent on covariates and confounders are not included in the scoring, resulting in a potential bias. Small sample size also introduces bias[28,55]. The focus on hospitalized patients can hamper the generalizability of milder and asymptomatic COVID-19 cases. Similarly, heterogeneous sample sizes impact data collection and yield inconsistent results. Socio-demographics, like age and gender, may also influence drug effects on severity and mortality.

Possible mechanisms underlying the findings

A hypercoagulative state is promoted by COVID-19-induced coagulative abnormalities and alterations in prothrombotic factors, such as enhanced factor VIII, plasma fibrinogen, microparticles, and increased platelet activation[86-89]. This hypercoagulative state, known as COVID-19-associated coagulopathy (CAC), differs from acute disseminated intravascular coagulation (DIC) in presentation, involving thrombosis instead of bleeding[90]. Although D-dimer levels are elevated in both CAC and DIC, additional coagulation factors differentiate CAC (higher levels of Von Willebrand factor (vWF) antigen, Factor VIII activity, and fibrinogen) from DIC (lower levels of fibrinogen, reduced Factor VIII activity, and decreased VWF)[88-91].

Evidence suggests coagulation factor Xa's role in virus entry by cleaving SARS Spike proteins, which certain anticoagulants inhibit, potentially affecting viral fusion with ACE-2 receptors[10]. Ageno *et al*[28] used this concept to show DOACs' potentially higher antiviral efficacy over VKAs, though with limitations. Meanwhile, in our meta-analysis, DOAC-treated cases showed more severe disease progression than VKA-treated ones.

The underlying pathophysiology of endothelial damage by SARS-COV-2 is thought to be related to the renin-angiotensin-aldosterone system (RAAS). Virus-host cell binding *via* ACE-2 receptors raises disease severity risk. Thus, ACEI and ARBs (RAAS inhibitors) could heighten disease severity by increasing ACE2 receptor expression[16]. Anticoagulants, not affecting ACE2 receptors, might explain the non-significant enhancement in disease severity, as seen in our meta-analysis with no prehospital anticoagulation-COVID-19 severity correlation.

Clinical implications and future directions

Our study has significant clinical implications. Monitoring coagulation function in ICU patients using repeated platelet count, prothrombin time, and D-dimer measurements is vital. Elevated serum D-dimer predicts VTE and is a prognostic tool for COVID-19 risk stratification[92,93]. High D-dimer or rapid respiratory decline may indicate suspected VTE. Middeldorp *et al*[62] noted significantly higher D-dimer levels in ICU-admitted COVID-19-infected patients, regardless of chronic prehospital anticoagulation. Our analysis found that chronic anticoagulation does not significantly reduce the risk of new thromboembolic events in COVID-19 patients. This suggests that prior anticoagulation does not protect against COVID-19-related thromboembolic events. Due to the controversial effects of anticoagulant therapy on COVID-19 severity, further studies are needed.

Strengths and limitations

This meta-analysis's strengths include a sizable sample size dispersed across several nations and moderate to high-quality studies included according to the New-Castle Ottawa Scale. However, there are notable limitations. First, the retrospective nature of most included research limits the generalizability of the conclusions, highlighting the need for expansive prospective investigations. Second, while adjusted estimates were prioritized in the original analysis, selection bias and the confounding impact typical of observational research cannot be completely eliminated. Furthermore, inconsistency existed in the definition of severity among the included articles. Lastly, data gaps regarding prehospital anticoagulation specifics—duration, indication, type, and dosage—in some studies impeded comprehensive analysis.

CONCLUSION

The current meta-analysis concludes that prehospital anticoagulation does not significantly correlate with reduced COVID-19-related thromboembolic events, enhanced survival, or lowered disease severity risk. This aligns with recent guidelines advocating prophylactic anticoagulant use in COVID-19 patients, irrespective of VTE risk. To gain deeper insights and robust evidence, we suggest well-designed prospective studies and randomized trials investigating the impact of prior anticoagulant usage on thromboembolic risk, mortality, and disease severity in COVID-19 cases. Furthermore, a thorough exploration of the reasons behind the limited efficacy of chronic anticoagulants in severe infections is warranted. Future research should focus on determining personalized VTE risks for COVID-19 patients, uncovering underlying pathogenic pathways, and identifying optimal anticoagulant interventions for VTE prevention.

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FOOTNOTES

Author contributions: Iqbal K and Bansal V contributed equally to defining the study outline and manuscript writing; Data review and collection were done by Banga A, Iqbal A, Ahmed J, Iqbal K, Sharma N, Mehdi M, Kumar P, and Rathore SS; Statistical analysis was done by Banga A, Iqbal K, and Bansal V; Study design and critical review are done by Banga A, Lal A, Domecq JP, Kashyap R, and Bansal V; Iqbal K and Bansal V are the co-corresponding authors of the paper, taking responsibility for the integrity of the work from inception to the published article; all authors contributed to the article and approved the submitted version. Bansal V and Domecq JP contributed equally to this work and are considered co- corresponding authors.

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Country of origin: United States

ORCID number: Kinza Iqbal 0000-0001-5460-4406; Akshat Banga 0000-0003-2626-8219; Taha Bin Arif 0000-0003-4879-560X; Sawai Singh Rathore 0000-0003-1954-094X; Abhishek Bhurwal 0000-0002-3886-7537; Syeda Kisa Batool Naqvi 0000-0002-2049-5739; Muhammad Mehdi 0000-0002-6799-2548; Pankaj Kumar 0000-0002-5580-9144; Mitali Madhu Salklan 0009-0002-1139-5725; Ayman Iqbal 0000-0002-0993-6396; Jawad Ahmed 0000-0001-5651-1916; Nikhil Sharma 0000-0002-3718-6967; Amos Lal 0000-0002-0021-2033; Rahul Kashyap 0000-0002-4383-3411; Vikas Bansal 0000-0001-6047-5559; Juan Pablo Domecq 0000-0002-8540-9862.

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LETTER TO THE EDITOR

Reckoning with COVID-19 denial: Brazil's exemplary model for global response

Heslley Machado Silva

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Heslley Machado Silva, Department of Science and Education, University Center of Formiga and State University of Minas Gerais, Minas Gerais 35570000, Brazil

Corresponding author: Heslley Machado Silva, PhD, Postdoc, Professor, Department of Science and Education, University Center of Formiga and State University of Minas Gerais, Av Doutor Arnaldo Sena 328, Minas Gerais 35570000, Brazil. heslley@uniformg.edu.br

Abstract

In the aftermath of the coronavirus disease 2019 (COVID-19) pandemic in Brazil, accountability is crucial for those who denied the severity of the virus, spreading false information and causing harm. Some individuals have already faced legal proceedings against them, revealing economic motivations behind their actions. It is equally important to hold doctors accountable for prescribing ineffective treatments, putting the population at risk. The leaders of the denial movement and the federal government, who mishandled the pandemic, should be held accountable for the high death toll. Seeking justice from the legislative and executive branches is necessary, along with exemplary measures for those who spread misinformation about COVID-19.

Key Words: Denialism; Responsibility; Mortality; COVID-19; Brazil

Core Tip: Amidst the tumultuous landscape of misinformation during the coronavirus disease 2019 pandemic, a Federal Court in Rio Grande do Sul, Brazil, has set a precedent by holding a group of doctors advocating unproven early treatments accountable for collective moral and health damages. The decision underscores the imperative to address the dissemination of false scientific information, emphasizing the need to prosecute not only medical practitioners but also those who exploit their social respectability to fuel anti-vaccine movements. The unfolding legal actions signal a critical juncture for justice and accountability, prompting reflection on the broader repercussions of scientific denialism on public health.

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TO THE EDITOR

It is necessary to settle the score

After everything that has occurred in Brazil during the Coronavirus disease 2019 (COVID-19) pandemic, and continues to occur on a smaller scale, it is time to settle the score with the deniers, those who, through scientific fake news, have caused deaths and suffering. The Federal Court in the state of Rio Grande do Sul has issued a decision against a group of doctors advocating for the so-called early treatment for COVID-19[1], which lacks scientifically proven effectiveness. These professionals have been ordered to pay compensation in the amount of R$ 55 million for collective moral and health damages. This decision was announced by the Federal Public Prosecutor's Office (MPF) on May 25, 2023.

The case of false information about electronic voting machines in the United States, disseminated by Fox News, illustrates that it is possible to hold accountable (even though this TV channel made a multi-million-dollar deal to settle the case) those who believe they can spread misinformation and not face any consequences; it is time to reflect and act on this matter. It is not too late to hold former President Bolsonaro accountable for his actions and speeches that resulted in deaths during the pandemic[2]. An example of his irresponsible practice was when he claimed on a social media platform, followed by millions of people, many of whom were misinformed or uninformed, that the COVID-19 vaccine could cause acquired immunodeficiency syndrome[3]. One can only imagine how many people refrained from getting vaccinated because of this fraudulent news and how many perished because of this and many other "live" events with such an approach, disseminated by the leader.

It is time to bring to trial a group of Brazilian scientists who signed a manifesto in favor of the use of harmless COVID-19 drugs and who opposed the use of vaccines[4]. It is also time to hold accountable journalists and other once respected professionals who used their supposed respectability to support COVID-19's anti-vaccine movement and in favor of dead-end drugs[5,6]. All those who somehow supported this movement, fomented the use of useless drugs have their share of blame, but one group in particular has a greater responsibility, the doctors, because after all there is a (correct) mantra widely spread in Brazil: "Never take any kind of medicine without a doctor's prescription"[7]. But what should the Brazilian population have done, when a considerable part of the medical community prescribed useless and dangerous drugs in the middle of a pandemic? The population went to the doctors and fell prey to anti-scientific obscurantism.

It is necessary to hold responsible those doctors, both in Brazil and around the world, who even after the proof that these drugs had no effect in combating the disease, continued to prescribe them due to political, ideological or religious affinity, abdicating any scientific basis in their practice[1,8]. Furthermore, it is necessary to criminalize the offenses committed by these doctors, since by prescribing these drugs early in the treatment, they only worsened the condition of patients with COVID-19, and these drugs could have been effective in other situations, later in the evolution of the disease.

But it is crucial to recognize that those who prescribed this dangerous cocktail preemptively committed much more serious crimes against their patients[9]. They poisoned healthy people, who were not sick, with dewormers, antibiotics [6], and antineoplastic drugs, among others. These patients, when they really required severe acute respiratory syndrome coronavirus 2 drugs, ended up more vulnerable and showed higher lethality and morbidity. At the beginning of 2024, new studies suggest that the tragedy caused by the misuse of chloroquine in the treatment of COVID-19 may have caused many more deaths than had been imagined[10].

Moreover, these health professionals not only exposed their own patients, but also put the entire population at risk. We must reflect that by prescribing antibiotics, for example, for a viral disease, or even worse, preventively in healthy individuals[9], the entire population is exposed to the risk of selection of resistant bacteria. The result is that antibiotics are rendered ineffective when really needed[11], with consequences that we cannot yet measure.

This historical moment, marked by the pandemic and the exponential growth of the spread of fake news[12], should serve as an opportunity for holding accountable those who have directly or indirectly caused the death and suffering of thousands or even millions of people. A pandemic with millions of deaths, occurring simultaneously with the increased influence of social networks and the dissemination of false scientific news, highlights the need to hold accountable those who contributed to this misinformation[13], taking advantage of its relative social respectability and knowledge of new media.

An interview with the Minister of Health of the Lula government, published on June 5, 2023, addresses the urgency of holding accountable denialist doctors who spread false news about vaccines on social networks. According to Minister Nísia Andrade, the misinformation by these doctors regarding vaccination will be investigated, and a working group will be formed composed of the judiciary and the executive to assess who will be held accountable and how this will be done. This speech demonstrates a positive change from the Brazilian federal government, in relation to the fight against Fake News, and points to the hope that this cycle of misinformation through health professionals will be interrupted in the country.

Justice is coming...

The Brazilian justice system has started to partly reach some groups, as mentioned before, but we must wait and see what the results will be. The federal court has judged two actions of the MPF against those responsible for publishing the material entitled "Manifesto Pela Vida". This group, which called itself "doctors of early treatment in Brazil", encouraged the use of drugs that were supposed to be an "early treatment" against COVID-19. This material was disseminated to the general population, including the indication of doctors who prescribed the so-called covid kit[14]. Médicos Pela Vida [Medical Dignity Association of Pernambuco (ADM/PE)], together with the companies Vitamedic Indústria Farmacêutica, Centro Educacional Alves Faria (Unialfa) and the José Alves Group (GJA Participações), were jointly condemned to pay R$ 55 million for collective moral damages and to health, within their responsibilities. In one of the actions, the amount imposed by justice was R$ 45 million, and in the other, the condemnation was R$ 10 million.

The financial relationship between these groups was confirmed, justifying the need for exemplary measures. A medical association in Recife (PE), composed of physicians registered with Cremers (Regional Council of Medicine of Rio Grande do Sul), publicized in an advertisement the supposed benefits of "early treatment" for COVID-19, explicitly mentioning the drugs used. However, this reference omitted the adverse effects of the drugs, potentially encouraging self-medication due to the medical association's recommendation. The collaboration between the pharmaceutical company Vitamedic and the Associação Médicos Pela Vida was proven, with the company irregularly financing this advertisement, investing R$ 717 thousand. This information was admitted by Vitamedic's director in testimony in the Parliamentary Inquiry Commission (CPI) of COVID in the Federal Senate, Vitamedic being the manufacturer of ivermectin. That company had an increase in its revenue from R$ 15.7 million in 2019 to R$ 470 million in 2020 just from the sale of ivermectin, one of the drugs ineffective against COVID-19. This case highlighted that many advocates of the so-called "kit-covid" were being funded to deceive the population, resulting in significant profits for companies like Vitamedic at the expense of the lives of thousands of Brazilians.

The leaders of this directed catastrophe have yet to be held accountable

The numbers show the harmful effects of the erratic handling of the pandemic by the federal government and its followers, mainly due to the dissemination of false news over the internet and social networks[15]. Brazil, with only 2.7% of the world's population, accounts for 10% of global COVID-19 deaths. By mid-February 2023, the death rate in the world was 860 deaths per million inhabitants, while in Brazil it was 3200 deaths per million inhabitants.

In Brazil, intubation proved to be more dangerous, with 80% of patients in this state dying, while the global average was 50%[16]. Why did this happen in this country? Brazil's healthcare system is not among the worst in the world[17], and its doctors and nurses are competent. However, it was one of the only countries that poisoned its population with dozens of useless drugs, such as the "kit-COVID"[18], that have proven side effects[19] making it absurd to prescribe them even preventively[9]. As evidence of this correlation, the municipalities most aligned with President Bolsonaro's thinking, which earned him the most votes, were those that registered proportionally higher numbers of COVID-19 deaths[20,21].

The consequences could have been even more damaging if all the vaccination efforts carried out within the Unified Health System had not occurred and if civil society and the CPI of the pandemic had not pressed for a reorientation of the Federal Government's actions. In addition, the Federal Supreme Court legally prevented the Bolsonaro government from harming municipalities and states by taking the necessary measures to contain the pandemic[22].

It is necessary that the entire Brazilian society, which has suffered the most from all this erratic science denial movement, demand justice from all the constituted powers, legislative and executive. All doctors and any other professionals who have spread and still spread false news about COVID-19 must be exemplarily disciplined, either financially or professionally (*e.g.*, losing their medical licenses). A country like Brazil, which has an internationally recognized public health system[23] despite its problems, and which has had exemplary vaccination rates, cannot abandon itself to the mercy of inconsistent and unscientific deniers.

Let us hope in the next health emergency that occurs - as it surely will, even if we don't know when - that we will only have to face the disease and its inherent difficulties, and not also problems created by professionals who fail to fight against, or who even fight for, the threat to the public health. May we also have governors who are guided by scientific rigor and who protect their population regardless of their ideology and personal convictions.

FOOTNOTES

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ORCID number: Heslley Machado Silva 0000-0001-8126-8962.

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