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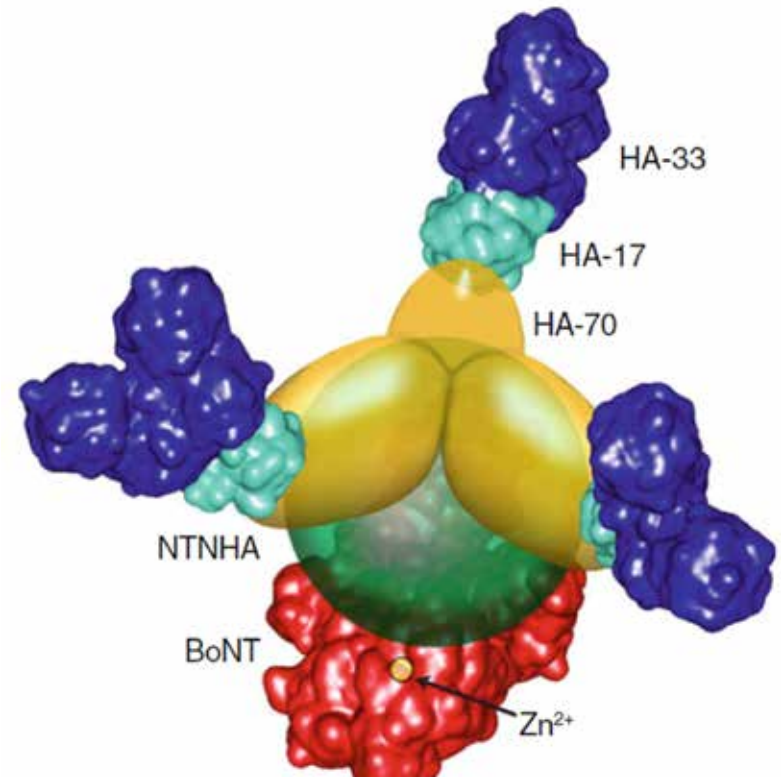

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Reference:
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KAZALO / CONTENTS

Pravni okvir umetne inteligence

Umetna inteligenca postaja realnost v današnjem svetu in se vključuje v vse več vidikov našega življenja. Razvoj novih tehnologij, ki delujejo na podlagi umetne inteligence predstavlja nesluten napredek, prinaša številne koristi in odpira mnoga vprašanja. Uporabi le te se ni več mogoče izogniti; potrebno se je z njo seznaniti, uporabiti njene prednosti in se hkrati zavedati izzivov, ki jih ta prinaša z rabo. Vpliv umetne inteligence gre zaznati na vseh področjih delovanja, tj. od proizvodnje, javne uprave, prometa do znanosti in izobraževanja, ter tudi v zdravstvu je na določenih področjih že močno prisotna. Za pravilno in varno uporabo je potrebno definirati ustrezen pravni okvir, ki bo postavil temelje za ustreznost delovanja in reševanje določenih pravnih vprašanj, ki se odpirajo na tem področju. Kot izhodiščni pravni okvir umetne inteligence bi lahko izpostavili s strani Generalne skupščine Unesca, tj. novembra 2021 izdan prvi globalni standard o etiki umetne inteligence „Priporočilo o etiki umetne inteligence“, ki ga je v letu izdaje sprejelo 193 držav članic Unesco. To je prvi mednarodno pravni instrument na področju etike v umetni inteligenci, ki je globalni okvir za vključujočo in odgovorno uporabo umetne inteligence v naših družbah. Zaščita človekovih pravic in dostojanstva je temelj Priporočila, ki temelji na napredku temeljnih načel, kot sta preglednost in pravičnost, pri čemer se vedno opominja na pomen človeškega nadzora nad sistemi umetne inteligence. Priporočilo je pripravljeno na način, da je dovolj obsežno, da oblikovalcem politik omogoča, da temeljne vrednote in načela prevedejo v dejanja v zvezi z upravljanjem podatkov, okoljem in ekosistemi, spolom, izobraževanjem in raziskavami, ter tudi zdravjem in socialno blaginjo ter številnimi drugimi področji. V 2023 je Svet Evropske komisije sprejel Evropsko deklaracijo o digitalnih pravicah in načelih za digitalno desetletje 2023/C 23/01, ki izpostavlja pojem digitalna preobrazba z vplivom na vse vidike življenja, pri čemer prinaša izzive za

Legal framework for artificial intelligence

Artificial intelligence is becoming a reality in today's world, and is becoming increasingly integrated into our lives. The development of new technologies based on artificial intelligence is unprecedented, bringing numerous benefits and raising many legal questions. Its use can no longer be avoided; we must familiarize ourselves with it, take advantage of its benefits, and at the same time, be aware of the challenges it brings. The impact of artificial intelligence can be felt in all areas of the economy from manufacturing, public administration, transport, science, and education. It is also strongly present in some areas of healthcare. For its proper and safe use, it is necessary to define an appropriate legal framework that will lay the foundation for its proper functioning and resolve legal issues that arise in in the field of artificial intelligence As a starting point for the legal framework for artificial intelligence, we could highlight the first global standard on artificial intelligence ethics, the "Recommendation on the Ethics of Artificial Intelligence," issued by the UNESCO General Conference in November 2021 and adopted by 193 UNESCO member states in the year of its publication. This is the first international legal instrument in the field of ethics in artificial intelligence, providing a global framework for the inclusive and responsible use of artificial intelligence in society. The protection of human rights and dignity is the foundation of the Recommendation, which is based on the advancement of fundamental principles, such as transparency and fairness, while always remembering the importance of human control over artificial intelligence systems. The recommendation is designed to be comprehensive enough to enable policymakers to translate fundamental values and principles into action, including in relation to data governance, the environment and ecosystems, gender, education and research, and health and social well-being, among others. In 2023, the Council of the European Commission adopted the European Declaration on Digital Rights and Principles for the

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demokracijo, gospodarstvo in posameznike. Za smernico je določeno, da naj bi bile digitalne javne storitve na spletu dostopne nemoteno in varno, zasnovane za učinkovito izpolnjevanje potreb ljudi, vključno z digitalnimi zdravstvenimi storitvami in storitvami oskrbe, predvsem z dostopom do elektronske zdravstvene dokumentacije. V okviru Evropskega parlamenta in Sveta je bila dne 13. junija 2024 sprejeta Uredba (EU) 2024/1689 o določitvi harmoniziranih pravil o umetni inteligenci in spremembi uredb (ES) št. 300/2008, (EU) št. 167/2013, (EU) št. 168/2013, (EU) 2018/858, (EU) 2018/1139 in (EU) 2019/2144 ter direktiv 2014/90/EU, (EU) 2016/797 in (EU) 2020/1828 (slednja imenovana Akt o umetni inteligenci). V Aktu o umetni inteligenci so izpostavljene tudi z umetno inteligenco omogočene manipulativne tehnike, ki se lahko uporabljajo za napeljevanje oseb k neželenemu vedenju ali za njihovo zavajanje, tako, da se jih spodbuja k odločitvam na način, ki spodkopava in ovira njihovo avtonomijo, odločanje in svobodno izbiro. Prepovedi takih praks dopolnjujejo določbe Direktive 2005/29/ES Evropskega parlamenta in Sveta, zlasti v smislu, da so nepoštene poslovne prakse, ki potrošnikom povzročajo ali gospodarsko ali finančno škodo, prepovedane v vseh okoliščinah, ne glede na to, ali se izvajajo prek sistemov umetne inteligence ali kako drugače. Prepoved manipulativnih in izkoriščevalskih praks iz te uredbe ne bi smela vplivati na zakonite prakse v okviru zdravljenja, kot je psihološko zdravljenje duševne bolezni ali telesna rehabilitacija, kadar se te prakse izvajajo v skladu z veljavnimi pravnimi medicinskimi standardi in zakonodajo, npr. izrecna privolitev posameznikov ali njihovih zakonitih zastopnikov. Izpostavljeni so avtonomni roboti, ki bi morali biti sposobni varno delovati in opravljati svoje funkcije v kompleksnih okoljih, še posebej v zdravstvenem sektorju, v katerem je tveganje za življenje in zdravje še posebej visoko, uporabljajo vse bolj izpopolnjene diagnostične sisteme in sistemi, ki podpirajo človeške odločitve, morajo biti zanesljivi in točni. Predpisano je prav tako, da bi bilo potrebno sisteme umetne inteligence, ki se uporabljajo za ocenjevanje in razvrščanje klicev v sili fizičnih oseb

Digital Decade 2023/C 23/01, which highlighted the concept of digital transformation affecting all aspects of life, posing challenges for democracy, the economy, and individuals. The guideline stipulates that digital public services should be securely accessible online without interruption and be designed to effectively meet people's needs, including digital health and care services, in particular, access to electronic health records. Regulation (EU) 2024/1689 harmonised rules on artificial intelligence, as did amending Regulations (EC) No 300/2008, (EU) No 167/2013, (EU) No 168/2013, (EU) 2018/858, (EU) 2018/1139 and (EU) 2019/2144 and Directives 2014/90/EU, (EU) 2016/797 and (EU) 2020/1828 (referred to as the Artificial Intelligence Act). The Act on Artificial Intelligence also highlights manipulative techniques enabled by artificial intelligence that can be used to induce individuals to engage in undesirable behavior or to mislead them by encouraging them to make decisions in a way that undermines and impedes their autonomy, decision-making, and free choice. Prohibitions on such practices complement the provisions of Directive 2005/29/EC of the European Parliament and of the Council, in particular, in the sense that unfair commercial practices causing economic or financial detriment to consumers are prohibited in all circumstances, regardless of whether they are carried out through artificial intelligence systems or otherwise. The prohibition of manipulative and exploitative practices in this Regulation should not affect legitimate practices in the context of medical treatment, such as the psychological treatment of mental illness or physical rehabilitation, where such practices are carried out in accordance with applicable legal medical standards and legislation, including the explicit consent of individuals or their legal representatives The Artificial Intelligence Act sets out autonomous robots are highlighted, which should be able to operate safely and perform their functions in complex environments, especially in the healthcare sector, where the risk to life and health is particularly high.

Increasingly sophisticated diagnostic systems and systems that support human decisions must be reliable and accurate. The Artificial Intelligence Act stipulates it is also stipulated that artificial intelligence systems

ali za pošiljanje oz. določanje prednosti pri napotitvi služb za ukrepanje ob nesrečah, vključno s policijo, gasilci in medicinsko pomočjo, ter sistemih triaže pacientov v nujnem zdravstvenem varstvu, razvrstiti med sisteme visokega tveganja, saj sprejemajo odločitve v zelo kritičnih razmerah za življenje in zdravje oseb ter njihovo premoženje. Da bi zagotovili ustrezen okvir za uporabo umetne inteligence na posameznih področjih, je bila sprejeta še vrsta pravnih regulacij in smernic, ki poskušajo opredeliti meje in načela za uporabo naprednih tehnologij; če izpostavim zgolj temeljno, tj. BELA KNJIGA o umetni inteligenci – evropski pristop k odličnosti in zaupanju, ter PRIPOROČILO KOMISIJE (EU) 2019/243 z dne 6. februarja 2019 o evropski obliki izmenjave elektronskih zdravstvenih zapisov.

Okvir nacionalne zakonodaje, ki bo postavil temelje za uporabo umetne inteligence v Republiki Sloveniji je Zakon o izvajanju Uredbe (EU) o določitvi harmoniziranih pravil o umetni inteligenci. Ministrstvo za digitalno preobrazbo je med cilje zakona zapisalo: spodbujanje inovacij in razvoj naprednih rešitev, ki služijo ljudem, ter hkrati varovanje zdravja, varnosti in temeljnih pravic posameznika. Izpostavila bi, da je to šele začetek, saj se dnevno pojavljajo vprašanja, kje in kako lahko umetna inteligenca pomaga, kaj sploh to pomeni, kje je njena učinkovitost največja in s kakšnimi odprtimi vprašanji se srečujemo pri njeni uporabi. Kot največjo uporabnost te sodobne tehnologije, se izpostavlja določena rutinska opravila, ki zahtevajo natančno analizo večjih količin podatkov, saj sodi v uporabno računalniško znanost, ki prek računalniških algoritmov izvede določena opravila. Umetna inteligenca se danes potemtakem lahko uporablja v različnih fazah postopkov zdravljenja, pri čemer vsaka faza vključuje različne specifične primere uporabe. Ta uporaba mora biti odgovorna in preverljiva, kot recimo analiza podatkov pacienta in pomoč pri odločanju o možnosti zdravljenja, ki pa mora temeljiti na odločitvi usposobljenih zdravniških timov in ne sme biti izključno odločitev algoritma. Problem lahko nastane, če se zdravniške odločitve sprejemajo izključno z uporabo algoritmov umetne inteligence. V zdravstvenem okviru uporaba

used to assess and classify emergency calls from individuals, or to dispatch or prioritize emergency services, including the police, firefighters, and medical assistance, as well as systems for triaging patients in emergency medical care, should be classified as high-risk systems, as they make decisions in situations that are critical to the life and health of individuals and property. To ensure an appropriate framework for the use of artificial intelligence in specific areas, a number of legal regulations and guidelines have been adopted that attempt to define the limits and principles for the use of advanced technologies, of which I would like to highlight the »White paperon artificial intelligence - the European approach to excellence and trust, and Commission recommendation« (EU) 2019/243 of 6 February 2019 on a European model for the exchange of electronic health records.

The framework of national legislation that will lay the foundations for the use of artificial intelligence in the Republic of Slovenia is the Act Implementing Regulation (EU) on the establishment of harmonized rules on artificial intelligence. The Ministry of Digital Transformation has set out the following objectives for the Act: to promote innovation and the development of advanced solutions that serve people, while protecting the health, safety, and fundamental rights of individuals. I would like to point out thatthis law is only the beginning, as questions arise daily about which areas the economy artificial intelligence could help, , where its effectiveness is greatest, and what open questions remain for its use. The greatest utility of this modern technology is highlighted in routine tasks that require precise analyses of large amounts of data, as it belongs to the field of applied computer science, which performs tasks using computer algorithms. Artificial intelligence can therefore be used in various stages of treatments, with each stage involving different specific use cases. Every use must be responsible and verifiable, such as analyzing patient data and assisting in determiningtreatment options, which must be based on the decisions of qualified medical teams, and not solely on the decisions of an algorithm.

A problem may arise when medical decisions are made solely through the use of artificial intelligence algorithms. In a healthcare setting, the use of artificial

tehnologije umetne inteligence prinaša dodatne pomisleke, saj medicinske odločitve ne bi smele temeljiti na avtonomnih sredstvih, ki jih zdravniki ne morejo razumeti. Izpostavlja se, da bi moral zdravnik, da bi zadostil standardu skrbnega ravnanja, vsako odločitev algoritma kritično presoditi, kar pomeni, da naj bi bil namen uporabe umetne inteligence razširitev in izboljšanje zdravnikovega sedanjega znanja, ne pa njegovo nadomeščanje.

Avtonomna orodja umetne inteligence bo torej potrebno vpeljevati premišljeno in predvsem z ustreznimi pravnimi rešitvami za odgovorno rabo ter v zaščito tako pacientov kot zdravstvenega osebja. Pri teh izzivih sta torej medicinska in pravna stroka izjemno povezani.

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intelligence raises additional concerns because medical decisions should not be based on autonomous means that doctors cannot understand. I should emphasize that to meet the standard of care, a doctor should critically evaluate every decision made by an algorithm, which means that the purpose of using artificial intelligence should be to expand and improve the doctor's current knowledge, not to replace it. Autonomous artificial intelligence tools will therefore need to be introduced in a considered manner, and above all, with appropriate legal solutions for responsible use and the protection of both patients and healthcare personnel. The medical and legal professions are therefore closely linked when it comes to such challenges.

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Faculty of Law, University of Maribor

Razvoj študija farmacije na Medicinski fakulteti Univerze v Mariboru: kronološki pregled do akreditacije leta 2025

Development of Pharmacy Education at the Faculty of Medicine, University of Maribor: A Chronologic Review Leading to the 2025 Accreditation

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Izvleček

Namen: *Vzpostavitev enovitega magistrskega študijskega programa Farmacija na Medicinski fakulteti Univerze v Mariboru (MF UM) je bila posledica nacionalnega pomanjkanja farmacevtov, širitve farmacevtske industrije, večjih potreb po klinični farmaciji ter usklajevanja z evropskima direktivama 2013/55/EU in 2024/782/EU. Ti dejavniki so potrdili nujnost drugega akreditiranega študijskega programa farmacije v Sloveniji. Članek obravnava razvoj, ki je decembra 2024 pripeljal do akreditacije programa.*

Metode: *Izveden je bil kronološki, na dokumentih temelječ pregled institucionalnih arhivov, treh akreditacijskih vlog, poročil Nacionalne agencije Republike Slovenije za kakovost v visokem šolstvu (NAKVIS), internih strateških analiz, partnerskih sporazumov ter nacionalnih statističnih podatkov. Viri so bili dopolnjeni z znan-*

Abstract

Background: *Establishment of the Integrated Master's Programme in Pharmacy at the Faculty of Medicine, University of Maribor (MF UM) was driven by national pharmacist shortages, expansion of the pharmaceutical industry, increasing clinical pharmacy needs, and harmonisation under EU Directives 2013/55/EU and 2024/782/EU. These factors underscored the necessity of a second accredited pharmacy programme in Slovenia. This review summarises the development that led to accreditation in December 2024.*

Methods: *A chronologic document-based review was performed using institutional archives, three accreditation applications, reports from the Slovenian Quality Assurance Agency for Higher Education (NAKVIS), internal strategic analyses, collaboration agreements, and*

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stveno literaturo s področja farmacevtskega izobraževanja in evropskega regulativnega okvira z namenom identifikacije strukturnih, pedagoških in vsebinskih elementov, ki so oblikovali končni program.

Rezultati: *Razvoj je potekal v treh fazah: zgodnja zasnova (2006–2016); dve akreditacijski vlogi (2017–2020), ki sta razjasnili vsebinske, kadrovske in kompetenčne vrzeli; ter celovita prenova programa (2021–2024), ki je vključevala interdisciplinarno sodelovanje med MF UM, Fakulteto za kemijo in kemijsko tehnologijo Univerze v Mariboru (FKKT UM), kliničnimi ustanovami in partnerji iz farmacevtske industrije. Končni kurikulum je usklajen z evropskimi kompetenčnimi zahtevami in vključuje biomedicinske, tehnološke, analitske ter klinične vsebine. Tako klinične kot tudi industrijske komponente so bile utrjene preko obsežne mreže partnerstev.*

Zaključki: *Program predstavlja pomemben napredek na področju farmacevtskega izobraževanja v Sloveniji. Odgovarja na potrebe hitrega in obsežnega razvoja farmacije v Sloveniji, krepi regionalni razvoj ter dopolnjuje obstoječe izobraževalne zmogljivosti. Vzpostavitev tega programa poudarja pomen strateškega načrtovanja in medsektorskega sodelovanja v sodobnem farmacevtskem izobraževanju.*

national statistical data. These materials were integrated with the scientific literature on pharmacy education and EU regulatory frameworks to identify structural, pedagogic, and contextual elements shaping the final programme.

Results: *Development of the Integrated Master's Programme in Pharmacy progressed through three phases: early conceptualisation (2006–2016); two accreditation attempts (2017–2020), which clarified curricular, staffing, and competency gaps; and a comprehensive redesign (2021–2024) involving interdisciplinary collaboration among the MF UM, Faculty of Chemistry and Chemical Engineering, University of Maribor (FKKT UM), clinical institutions, and pharmaceutical industry partners. The final curriculum aligns with EU competence requirements and integrated biomedical, technological, analytical, and enhanced clinical sciences. Strong inter-faculty collaboration and robust clinical and industrial components were formalised through extensive partnerships.*

Conclusions: *The programme represents a significant advance for Slovenian pharmacy education, addressing national workforce needs, strengthening regional development, and complementing existing educational capacities. Establishment of the Integrated Master's Programme in Pharmacy at the MF UM highlights the value of strategic planning and cross-sector collaboration in contemporary pharmacy education.*

INTRODUCTION

Pharmacy, as a discipline in healthcare, has evolved rapidly at the intersection of biomedical innovation, clinical transformation, and expansion of the pharmaceutical industry. Over the past 2 decades the rapid development of biological and biotechnological medicinal products, the emergence of advanced therapy medicinal products, and the growing complexity of pharmacotherapy have expanded the scope and depth of competencies required of pharmacists (1). These shifts have paralleled the changes in European legislation, first with Directive 2013/55/EU and more recently with Directive 2024/782/EU, which together define an integrated profile of professional knowledge, skills, and attitudes, essential for the regulated profession of pharmacists across the European Union.

Until recently Slovenia maintained a single national pharmacy program at the University of Ljubljana. Despite the established reputation and academic strength, structural limitations (primarily related to space, staffing, and regional accessibility) prevented expansion of enrolment to meet the national need of pharmacists. At the same time, the Slovenian pharmaceutical sector underwent a significant transformation. Lek d.d. (part of Sandoz AG) initiated large-scale biopharmaceutical investments exceeding

€400 million, while Krka d.d. expanded its production and research footprint. In addition, several small and medium enterprises and technology-based companies intensified the demand for highly trained graduates. These developments highlighted the pressing need for a larger and more diverse cohort of pharmacists with strong competencies in industrial pharmacy, biotechnology, analytics, and regulatory science.

Concurrently, healthcare needs have also increased nationwide. Clinical pharmacy, once an emerging field in Slovenia, has expanded into hospital wards, specialised clinics, and interdisciplinary care teams. Numerous studies have demonstrated the increasing impact of clinical pharmacists on patient safety, therapy optimization, and the reduction of adverse drug events (2). Yet the capacity to train pharmacists with advanced clinical competencies in Slovenia is constrained.

The epistemic conjuncture (Figure 1) that framed the establishment of the pharmacy programme at the Faculty of Medicine, University of Maribor (MF UM) arose from the following convergence:

1. scientific and technological shifts in medicinal product development, particularly in biopharmaceuticals and advanced therapies;
2. clinical and healthcare system demands for pharmacists with stronger clinical integration;
3. industrial expansion, especially in northeastern Slovenia, requiring specialised competencies;
4. regulatory harmonisation under EU directives demanding comprehensive, unified pharmacist training;
5. the challenge of geographical equity and improving access to higher education for students from the eastern cohesion region, thereby addressing long-term regional development objectives; and
6. long-term strategic vision at MF UM, which has already successfully developed and implemented integrated programmes in medicine and dental medicine.

Recognising these pressures and opportunities, MF UM initiated a long-term project to establish a new Integrated Master's Programme in Pharmacy (Enoviti Magistrski Študijski Program Farmacija (EMŠP Farmacija)). While early conceptual discussions date back nearly 2 decades, systematic development intensified in 2016 with the formation of the first expert committee (3), followed by a sequence of structured accreditation attempts and comprehensive programme redesigns. The process culminated in December 2024 when the National Agency for Quality in Higher Education (NAKVIS) granted

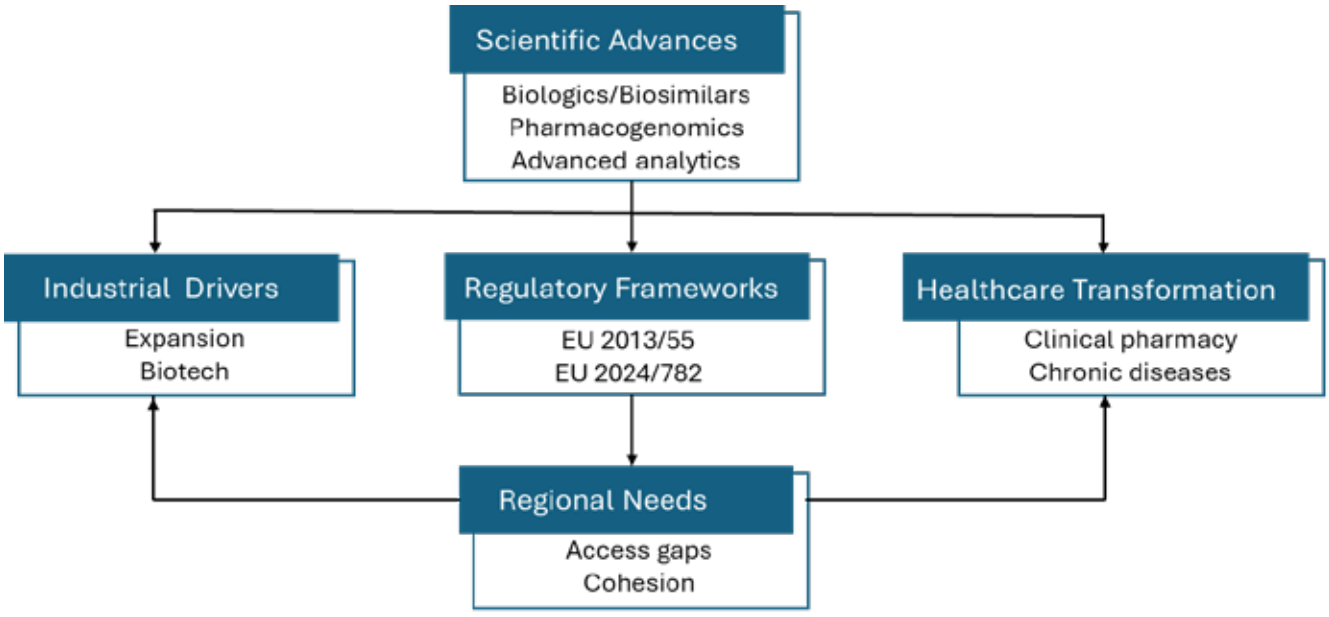

Figure 1. *Epistemic conjuncture influencing establishment of the programme.*

full accreditation (4), enabling enrolment of the first cohort of students in the 2025/2026 academic year. This article provides a comprehensive scholarly review of the process involved in developing the Integrated Master's Programme in Pharmacy at the MF UM. Building on institutional documentation, accreditation records, strategic analyses, and supporting literature, the chronologic evolution of the programme is reconstructed, the factors driving the programme design are critically evaluated, and the "Maribor model" is situated within national and European trends in pharmacy education. The review also highlights the unique interdisciplinary character of the programme, which integrats biomedical, chemical, technological, and clinical sciences across multiple UM faculties and external partners (5), (6).

The structure of this article follows a chronologic narrative, where the subsequent chapters outline all the developmental steps, from the earliest conceptual foundations (2006–2016) through several accreditation attempts and related curriculum redesigns (2017–2020), to the final successful accreditation (2021–2024). The article then discusses the regional, national, and European significance of the programme, concluding with reflections on future directions.

Together, these chapters provide insight into how institutional initiative, regulatory processes, and broader societal dynamics converged to establish the second accredited pharmacy programme in Slovenia.

HISTORICAL DEVELOPMENT AND EARLY FOUNDATIONS (2006—2016)

The idea of developing a pharmacy programme at the MF UM can be traced back to the establishment of the faculty nearly 2 decades ago. The MF UM benefited from strong natural, infrastructural, and academic conditions from inception that made the long-term development of pharmacy education feasible and strategically aligned with its mission. Very early discussions reflected in the first conceptual outlines of Lekarniška Farmacija and later Klinična Farmacija, indicate that the idea was present from the formative years of the institution (7).

Although the vision of establishing a pharmacy programme in Maribor had existed for many years, realization of the programme was demanding precisely because the development at the MF UM had to begin from scratch. Despite having well-established laboratory infrastructure in both the pre-clinical and clinical domains, the faculty initially lacked the critical mass of specialized experts in pharmaceutical sciences, which is an indispensable prerequisite for delivering a high-quality program in analytical, technological, clinical, and regulatory pharmacy. This initial absence of a broader, dedicated academic and research core necessitated a long-term, strategic, and highly coordinated process of building new teaching capacity, strengthening scientific disciplines, and establishing a stable interdisciplinary environment that spanned pharmaceutical technology, industrial pharmacy, clinical pharmacy, and related biomedical fields. These circumstances explain why the path to establishing the programme was significantly longer and more complex than the development of existing medical programmes. Today, after nearly 20 years of deliberate planning, recruitment, and close collaboration between the MF UM, FKKT UM, and numerous clinical and industrial partners, the original state of "beginning from scratch" has evolved into a mature academic ecosystem capable of delivering a comprehensive and modern pharmacy curriculum.

The concept of a pharmacy programme matured in parallel as the faculty grew across all core dimensions (professional, pedagogic, and especially scientific). Rapid expansion of interdisciplinary research, the recruitment and development of highly qualified staff, and pioneering contributions in emerging biomedical fields created an environment in which pharmaceutical education could naturally take root (8), (9). Comparable developments were occurring nationally and internationally because pharmacy, as a healthcare discipline, experienced substantial scientific and professional advances, including the rise of biopharmaceuticals, strengthened clinical pharmacy practice, and the increasing integration of pharmacists into healthcare systems (10).

These broader trends reinforced the relevance and timeliness of establishing a modern, interdisciplinary pharmacy programme at the MF UM, laying the conceptual foundation for the structured

development that would follow in subsequent years. Although informal discussions predate official documentation, the earliest structured records indicate that the first conceptual outline, under the working title "Lekarniška Farmacija," was actively explored until 2012–2013 (7). At that stage, the MF UM was still a relatively young medical institution, focused primarily on developing programmes in general medicine and later dental medicine. Nevertheless, several faculty members recognized the strategic opportunity to expand into pharmaceutical education, particularly due to the strong biomedical foundation of the faculty and emerging collaborations with clinical institutions.

During these early years, the principal motivations for exploring a pharmacy programme stemmed from the following:

1. increasing national need for pharmacists;
2. early recognition of regional imbalance in access to pharmacy education;
3. opportunities to leverage the clinical environment at the University Medical Centre Maribor (UKC Maribor); and
4. the long-term academic vision of the MF UM to integrate biomedical, clinical, and pharmaceutical sciences.

Although the initial concept was not fully developed, it already hinted at a model distinct from the only other study programme in pharmacy at the University of Ljubljana, one that would be more closely integrated with clinical practice and tailored to the specific needs of northeastern Slovenia.

By 2016 the MF UM formalised its intentions by shifting the conceptual framework to a new working title: "Klinična Farmacija" (8). This change reflected a strategic repositioning aligned with contemporary global and European trends that increasingly emphasised the clinical role of pharmacists (11). The MF UM possessed a unique advantage in this domain. Specifically, many of the faculty members also held clinical positions at UKC Maribor and the hospital already maintained a strong culture of interdisciplinary clinical collaboration. These circumstances allowed the concept of pharmacy education to mature, focusing on the integration of biomedical, patient-centered, and clinically oriented competencies.

A key milestone occurred in March 2016 when the MF UM appointed the first formal Expert Committee to prepare the programme (3). This committee established an institutional mandate to begin curriculum development, assess resource needs, and initiate consultations with external partners. The MF UM began signing cooperation agreements with clinical partners, public institutions, and early industry collaborators during 2016–2017 (6), demonstrating a growing recognition that pharmacy education must be integrated across healthcare and industrial environments.

Although these steps did not produce a complete curriculum, the foundation was laid for the distinctive identity that would ultimately define the MF UM study programme, one characterised by clinical proximity, interdisciplinary cooperation, and strong ties to practice-based environments.

FIRST AND SECOND ACCREDITATION ATTEMPTS (2017–2020)

The First Accreditation Application (2017–2019)

The formal development of the Integrated Master's Programme in Pharmacy accelerated in late 2017 when the MF UM submitted a request to the university leadership for permission to begin the accreditation process for the programme under its final name (EMŠP Farmacija) confirmed by the MF UM Senate in November 2017 (12). A unified application was finalised and approved by the UM Senate after internal alignment across involved faculties in June 2018 (13). The first full accreditation application was formally submitted to NAKVIS in June 2018 (14). Later that year (October 2018) NAKVIS appointed its Evaluation Committee, which subsequently conducted an on-site visit in February 2019 (15). The visit involved inspections of laboratories, discussions with faculty, and evaluation of the pedagogic and infrastructural readiness of the MF UM.

Following the visit, NAKVIS requested substantial revisions to the initial application (16), which the MF UM provided in April (17). Despite notable improvements, the final evaluation report delivered in

June 2019 (18) concluded that additional development, particularly in staffing depth and curriculum detail, would be necessary. The Ministry of Health issued a separate positive opinion on the programme at approximately the same time (19), confirming the national need for an additional pharmacy programme. However, the opinion was received after the NAKVIS report and could not influence the evaluation outcome. Considering the weight of the outlined shortcomings in the obtained reports, especially related to the staff, UM withdrew the first application from the accreditation process in August 2019 (20). Although disappointing, this withdrawal proved strategically constructive and provided an opportunity to rethink several key structural components of the programme, expand the academic base, and establish more robust clinical and industrial partnerships.

The Second Accreditation Application (2019–2020)

The MF UM submitted a second accreditation application (21) in October 2019 after several months of extensive work on the exposed issues. The MF UM prepared a much-needed upgrade to the documentation based on the feedback obtained from NAKVIS and a more refined vision. Early in 2020 NAKVIS appointed a new Evaluation Committee, which conducted a review and released an interim report in June 2020 (22).

The MF UM responded to all comments provided in July 2020 (23), improving several components of the programme, including curriculum alignment, staffing plans, and the integration of clinical pharmacy content. Nevertheless, indications from the evaluation process suggested that the second application was also likely to receive an unfavourable decision. Based on this information, and through our continuous commitment to ensuring long-term success rather than short-term approval, UM (again) strategically withdrew the application before the final assessment at NAKVIS (24).

This second withdrawal marked a critical turning point. Rather than continuing to iterate on the programme, which was incrementally improving but faced some structural limitations, the MF UM chose a strategic course of action (a comprehensive redesign of the programme). The faculty recognized that contemporary pharmacy education necessitated an even broader, future-oriented vision (deeper interdisciplinarity, stronger integration of industrial trends (biopharmaceuticals, analytics, and quality systems), substantially enhanced clinical training, and a sustainable long-term staffing model).

This strategic decision, favouring comprehensive restructuring over incremental concession, paved the way for the reimagined and ultimately successful final application.

THE FINAL ACCREDITATION APPLICATION AND PROGRAMME DESIGN (2021–2024)

Renewal of Strategy and Formation of the Extended Working Group (2021)

The MF UM undertook a comprehensive reassessment of its approach to pharmacy education following the strategic withdrawal of the second accreditation application. The faculty officially established a new extended working group with a broader academic, clinical, and industrial representation in July 2021 (25). This group included experts from the MF UM, FKKT UM, and UM, while internationally recognized scholars, clinicians, and industry representatives provided support as outside partners and supervisors. The establishment of this group marked a decisive shift from incremental corrections to systemic curricular redesign. The mandate included the following:

- full reconstruction of the curriculum aligned with the emerging new EU directive (2024/782/EU);
- reorganisation of subject areas to ensure improved vertical integration, considering the increasing competencies in all core pillars of pharmacy (apothecary work, clinical pharmacy, and industrial pharmacy);
- putting additional emphasis on strengthening the industrial, analytical, and pharmaceutical-technological components;
- deepening of clinical exposure and interdisciplinary training, considering the latter gaining importance in the European and Slovenian healthcare system; and
- development of a sustainable, long-term staffing plan.

Although the newly appointed working group had a

central role in shaping the successful final application, the majority of the substantive material, conceptual groundwork, and supporting documentation had already been generated during the previous two accreditation cycles. Over the following 3 years, the group systematically reviewed, consolidated, and reinterpreted this extensive body of earlier work, carefully integrating the work with the latest developments in pharmaceutical sciences, healthcare practice, and higher-education policy. In doing so, the group produced updated internal documentation, revised concept papers, refined competency matrices, and a restructured curricular framework, transforming previously accumulated content into a coherent, future-oriented programme.

This process involved not only aligning the existing materials with emerging European guidelines and best practices, but also "purifying" and reorganising the textual and structural components of the curriculum to better reflect contemporary professional expectations. Special attention was given to ensuring that the curricular architecture supported the progressive acquisition of key competencies across all major pillars of pharmacy, community/apothecary practice, clinical pharmacy, and industrial pharmacy, while also accommodating the expanding academic staff profile and interdisciplinary strengths. The result was a refined, pedagogically integrated, and strategically updated programme that retained the strongest elements of earlier versions, while incorporating essential modernisations required for a competitive, EU-aligned pharmacy curriculum.

Integration with FKKT UM and Expansion of Interdisciplinary Collaboration

An important step in the new programme design was the formal inclusion of the FKKT UM as a co-applicant for the programme in 2023 (5). This decision significantly strengthened the chemical and analytical foundations of the curriculum, aligning the curriculum with modern requirements for pharmaceutical chemistry, drug analytics, industrial processes, and quality assurance.

The collaboration was expanded substantially during this period within the UM and beyond. The deepened partnership with the FKKT UM, whose scientific productivity and long-standing excellence in analytical, physical, and organic chemistry, as well as chemical engineering and industrial process development based on strong collaboration with industrial partners, provided important disciplinary and strategic support for the programme. Involvement of the FKKT UM ensured an even more robust scientific foundation and helped align the curriculum with the expectations of modern pharmaceutical development, quality assurance, and analytical practices.

In parallel, the network of external collaborators, which had existed in earlier stages of programme development, was significantly expanded and formalised. Internationally recognised experts from universities in Graz and Sarajevo, contributed specialised knowledge in domains ranging from advanced analytics-to-pharmaceutical technology. At the same time, the MF UM deepened cooperation with clinical pharmacists, physicians, and researchers from UKC Maribor and regional hospitals, thereby strengthening the clinical relevance of the programme and ensuring that the curriculum reflected contemporary healthcare needs.

This period was marked not merely by the expansion of partnerships but by the concretisation of collaboration agreements, active involvement of specific academic and professional stakeholders, and establishment of clear commitments regarding teaching, mentorship, and research participation. These developments contributed to the refinement and optimisation of the final accreditation application, ensuring that the programme was supported by a cohesive and well-coordinated network of institutional, clinical, industrial, and international partners, whose expertise and engagement reinforced academic integrity and practical relevance.

The programme thus developed into a multi-faculty and -institutional educational ecosystem, positioning the MF UM and FKKT UM as highly competitive collaborators in pharmacy education.

Strengthening Industry Partnerships and Clinical Integration

The MF UM signed or renewed numerous cooperation agreements (collaboration contracts) with pharmaceutical and biomedical partners between 2021 and 2024. Formal endorsements were also secured from the two principal professional bodies in the field of pharmacy in Slovenia (the Slovenian Pharmaceutical Society and the Chamber of Pharmacy), further confirming the national relevance and professional support for the establishment of the programme (26), (27). Central among the professional bodies was the partnership with Lek d.d., which was formalised through two umbrella collaboration contracts covering educational and research activities. These agreements established a comprehensive framework for meaningful collaboration between the university and the industrial partners, providing students with opportunities to conduct thesis projects directly within pharmaceutical and biotechnological environments, where students could engage with real-world production, formulation, and analytical challenges. The MF UM gained the foundation for structured access to practical knowledge (e.g., production lines, quality-control laboratories, and advanced analytical platforms) through these partnerships, enabling students and academic staff to experience the technological and regulatory dimensions of pharmaceutical development firsthand. The cooperation also facilitated the systematic integration of good manufacturing practice (GMP) principles (which were already partially addressed by the FKKT UM), quality management, and regulatory science into the curriculum, ensuring that graduates would be well-prepared for contemporary industrial demands. Equally important was the active involvement of industry specialists in selected teaching activities, which brought current professional experience, case-based instruction, and technologically relevant perspectives into the academic setting.

Importantly, the formal agreements did not merely express an intention to collaborate but explicitly defined the modalities of cooperation in educational and research domains. On the educational side, public pharmacy institutions committed to providing structured training within the community-pharmacy setting, including clearly defined numbers of available mentors for practical training and sustained participation in the organisation of pharmacy practice. Industrial partners, in turn, agreed to contribute to specific curriculum components, particularly in courses covering analytical methods, pharmaceutical technology, regulatory affairs, and industrial manufacturing processes. On the research side, the agreements established a framework for joint scientific activities, outlining the basic conditions for collaborative projects, student research involvement, and the exchange of expertise and laboratory resources.

At the same time, clinical integration was significantly strengthened. Collaboration with UKC Maribor provided access to diverse clinical departments and interdisciplinary teams, allowing the development of practice-oriented courses in clinical pharmacology, pharmacotherapy, and clinical pharmacy. Partnerships with public pharmacy institutions in Maribor, Ptuj, and Ormož further expanded the practical foundations of the programme, offering authentic insights into community pharmacy operations, patient counselling, and primary healthcare workflows. Additional cooperation with specialised hospitals in Murska Sobota, Ormož, and Vojnik enriched the clinical landscape by adding settings with distinct therapeutic focuses and patient populations. Together, these clinical and community-based collaborations formed a cohesive network that ensured the

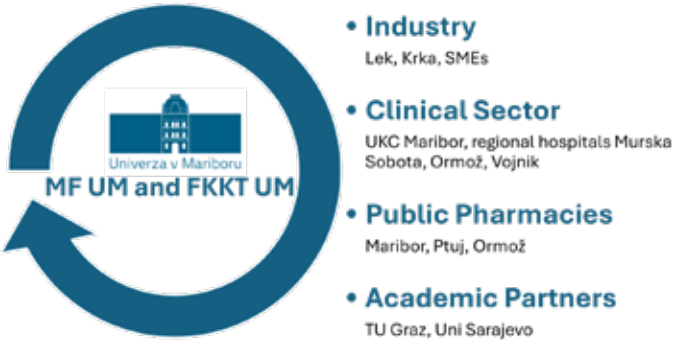

Figure 2. *Partner network map.*

programme was deeply embedded in the realities of Slovenian healthcare practice and that students would be exposed to a broad range of professional environments throughout their education.
All in all, this combined network enabled the creation of a uniquely practice-oriented curriculum within Slovenia (Figure 2).

Curriculum Architecture and Competence Framework

The final curriculum was designed in full alignment with the competence framework required by EU directives and international best practices in pharmacy education, integrating the structural, pedagogic, and professional expectations outlined in the accreditation documentation (28). The latter included the most relevant subject catalogue to date, as well as highlighting an updated list of crucial, future-oriented general and subject-specific competencies. The architecture follows a vertically integrated 5-year progression that systematically guides students from foundational biomedical and natural sciences toward increasingly specialised pharmaceutical disciplines, and ultimately, into advanced clinical and industrial practice. The early years of the programme emphasize the molecular, cellular, biochemical, anatomic, and physiologic foundations, ensuring that students acquire the scientific literacy necessary to understand drug action and therapeutic decision-making. Building on this foundation, subsequent years introduce the core pharmaceutical sciences, including pharmaceutical chemistry, technology, pharmacology, biopharmacy, and related analytical disciplines, allowing knowledge to develop in a structured and cumulative manner, as required by the principles of competency-based pharmacy education (29).
A defining characteristic of the curriculum is the pronounced approach towards gaining clinical competencies, reflecting both European trends and the longstanding clinical profile of the MF UM. Courses in clinical pharmacology, pharmacotherapy, social pharmacy, and clinical pharmacy are positioned within the heart of the curriculum and supported by direct integration with hospital-based teaching environments. This structure enables students to develop patient-centred competencies, such as communication skills, counselling, medication optimisation, and interprofessional collaboration, which are central to modern pharmaceutical care.
Equally important is the technological and industrial dimension of the programme, shaped by the growing practical orientation of the MF UM, the expertise of the FKKT UM, and the involvement of industrial partners. This is possible through a reimagined integration and alignment of modules, including pharmaceutical technology, pharmaceutical analysis and instrumentation, quality and stability of medicines, industrial pharmacy, and bioanalytics, all of which are continuously evolving fields, requiring direct connections with the practical environment. The industrial component ensures that students gain an understanding of GMP, regulatory processes, quality assurance, analytical evaluation of medicinal products, and the technological underpinnings of contemporary drug development. These elements reflect evolving global trends and the needs of the rapidly expanding pharmaceutical sector in Slovenia.
In addition to the clinical and industrial dimensions, the curriculum places strong emphasis on community pharmacy, which forms the third foundational pillar of the programme. Through subjects, such as social pharmacy, pharmaceutical care, communication in healthcare, and primary care pharmacotherapy, students acquire the competencies required for safe and effective practice in public pharmacies. This includes developing skills essential for medication counselling, managing minor ailments, supporting chronic disease management, and contributing to public health initiatives. The integration of community pharmacy training is further reinforced by collaborations with regional public pharmacy institutions, which provide structured practical experience and mentorship, ensuring that graduates are fully prepared for the professional realities of primary pharmaceutical care.
Interdisciplinarity, another central pillar of the programme, emerges naturally from the convergence of biomedical, chemical, technological, and clinical sciences. Subjects related to biopharmacy, pharmacogenomics, biotechnology, genetics, and biomedical informatics serve as integrative bridges connecting scientific foundations with therapeutic

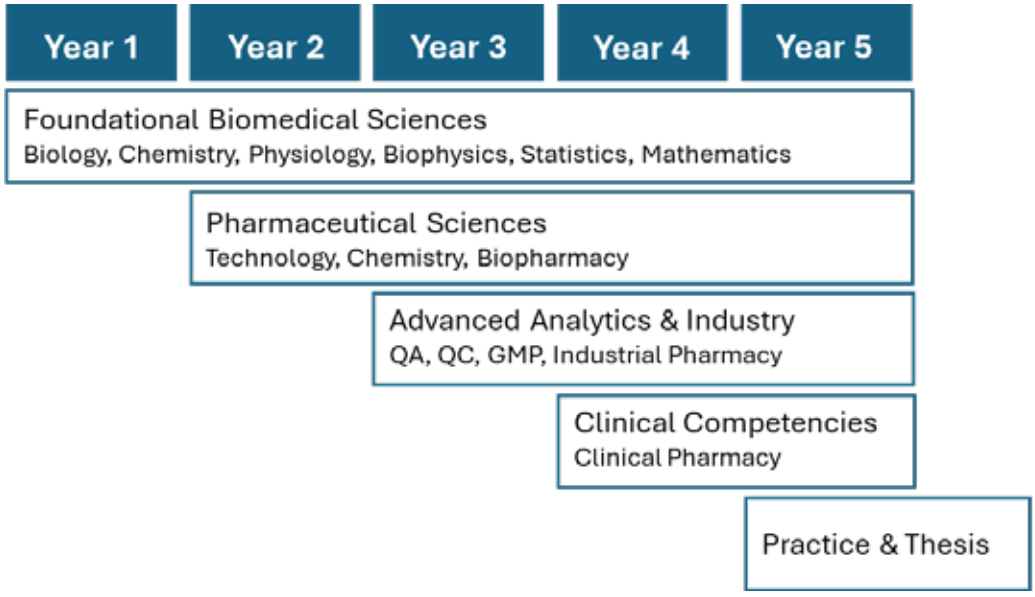

Figure 3. *Vertical integration of competences.*

and technological applications. Placement within the curriculum reflects the epistemic landscape identified in the accreditation application, where advances in molecular medicine, omics technologies, and digital health increasingly shape the role and expectations of pharmacists.

Taken together, the curriculum forms a coherent, competence-driven structure that supports the gradual acquisition of scientific, clinical, and technological knowledge, culminating in a comprehensive practical training experience and the preparation of a master's thesis in the final year. The design adheres not only to EU regulatory requirements but also to the broader strategic visions of the MF UM and FKKT UM, as articulated in the accreditation documentation, positioning graduates to contribute effectively across all major pillars of pharmacy (community, clinical, and industrial practice). Figure 3 illustrates the vertical integration of competencies acquired by students over the course of the 5-year programme.

Submission and Accreditation (2024)

After 3 years of intensive preparation and official withdrawal of yet another version of the documentation in December 2022 (30), the MF UM submitted the 4th and final accreditation application in February 2024. The NAKVIS Evaluation Committee provided a favourable interim report in June 2024 (31), followed by confirmatory updates and approvals within the MF UM, FKKT UM, and the University Senate in September 2024 (32). The Ministry of Health also approved this latest version of the study programme (33).

NAKVIS issued a highly positive final report in November 2024 (34). The NAKVIS Council formally granted accreditation on 19 December 2024 (35), marking the successful conclusion of a multi-year process. Subsequently, the Ministry for Higher Education confirmed funding for the first cohort through an official letter in January 2025 (36), enabling the UM to finalize all remaining organizational steps for the official launch of the programme in the autumn of 2025. This milestone enabled the enrolment of the first generation of pharmacy students in the 2025/2026 academic year, marking a historic moment for the MF UM, FKKT UM, and the broader University.

The newly admitted students successfully completed the introductory orientation day on 30 September 2025, during which an exceptionally positive atmosphere and strong sense of collegiality quickly

emerged among the pharmacy students and in the interactions with peers from other study programmes at the MF UM. This early display of mutual support and collaboration reflected the integrated academic culture that the MF UM had sought to cultivate throughout the development of the programme.

Equally encouraging was the excellent cooperation between tutors from the MF UM and the FKKT UM, who had a central role in guiding the new cohort through the initial academic and administrative transitions. The joint engagement demonstrated the strength of the interfaculty partnership underpinning the curriculum and provided a solid foundation for future interdisciplinary collaboration in teaching and mentoring. Figure 4 shows the timeline of the study programme development at MF UM from the start to accreditation in 2025.

The official start of the academic year on 1 October 2025 was accompanied by media coverage, including a feature on RTV Slovenia and segments on Radio Maribor, underscoring the regional and national importance of establishing the second pharmacy programme in Slovenia and highlighting the expanding role of the UM in pharmaceutical education.

REGIONAL, NATIONAL, AND EUROPEAN SIGNIFICANCE OF THE PROGRAMME

Slovenia has faced a persistent shortage of pharmacists for many years, a trend well documented by EUROSTAT, which consistently positions the country near the lower end of the European scale in terms of pharmacists per capita, the Statistical Office of the Republic of Slovenia that reports a steady 5%–10% annual growth in employment demand (37). This structural gap has been particularly evident in regions outside the central part of the country, where access to some fields in higher education is more limited and graduates are less likely to relocate. The UM directly addressed this imbalance and enhanced the availability of highly trained professionals in areas where professionals are most needed by establishing a pharmacy programme in Maribor. The presence of a second national centre of pharmacy education thus strengthened not only workforce capacity but also regional development and equity, supporting long-term cohesion objectives nationally and within the broader European framework.

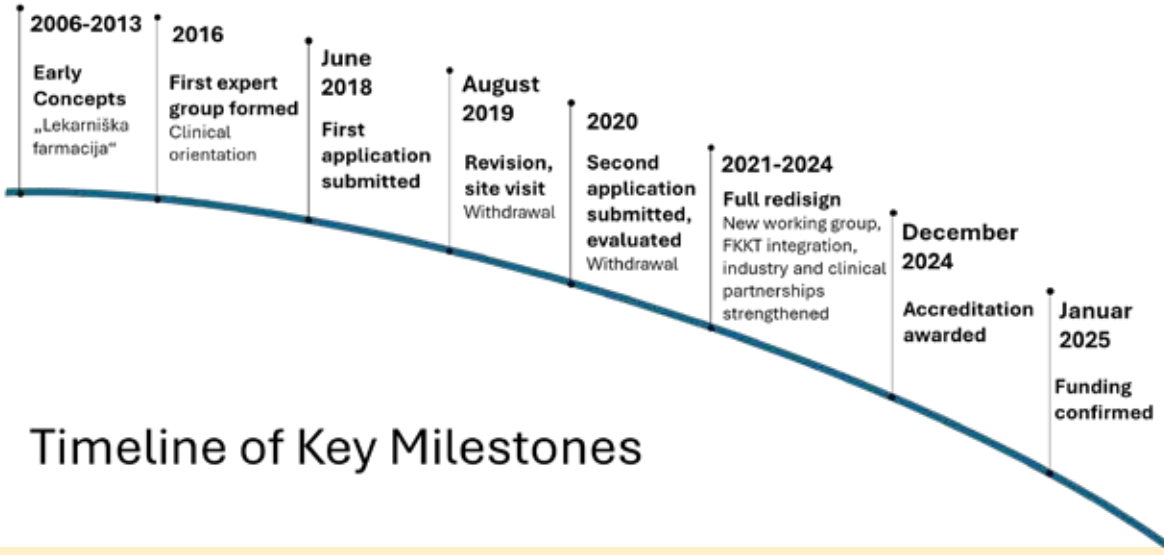

Figure 4. *Timeline of key milestones (2006–2024).*

The eastern cohesion region of Slovenia has historically been underrepresented among pharmacy graduates, largely due to financial, logistical, and social barriers associated with relocating to Ljubljana for study (38). The introduction of the programme at the MF UM fundamentally changed this landscape by providing a high-quality educational pathway within the region. This improved accessibility ensures that talented students who might otherwise have been deterred can now pursue pharmacy studies closer to home, contributing to greater regional retention of skilled professionals and reinforcing local healthcare and industrial capacity.

At the same time the programme responds to the rapid expansion and transformation of the Slovenian pharmaceutical industry, which has intensified investment in biotechnological development, advanced analytics, biosimilar production, and high-throughput manufacturing in recent years (39). Large-scale industrial projects, including major expansions by leading national pharmaceutical companies, as well as the growth of specialised SMEs in the wider Maribor region, have significantly increased the demand for graduates with strong technological, analytical, GMP, regulatory, and bioprocessing expertise. Through close collaboration with the FKKT UM and key industrial partners, many of whom have decades-long traditions of applied research and development, the curriculum integrates these industrial trends into the scientific and pedagogical framework. This approach not only ensures that graduates are prepared for the contemporary needs of the industry but also strengthens the innovation capacity across the pharmaceutical sector in Slovenia.

Equally important is the programme emphasis on clinical pharmacy and integration within the wider healthcare system. The proximity of the MF UM to UKC Maribor enables a depth of clinical exposure that aligns with European developments, emphasizing patient-centered pharmaceutical care, antimicrobial stewardship, personalized medicine, and multidisciplinary therapeutic collaboration. The curriculum dedicates substantial space to clinical pharmacology, pharmacotherapy, and clinical pharmacy, ensuring that students acquire the clinical reasoning, communication skills, and interprofessional competencies required for modern clinical roles. These elements are further reinforced through cooperation with public pharmacies and regional hospitals, offering students a comprehensive understanding of the continuum from community-to-clinical practice.

The »Maribor programme« complements, rather than competes with, the long-established pharmacy programme in Ljubljana. The demographic trends in Slovenia, combined with industrial expansion and increasing healthcare demands, require more than a single educational provider. The strengths of the MF UM in clinical sciences and biomedical technology, the FKKT UM in chemical engineering and various branches of chemistry, and the broader institutional culture of interdisciplinarity in the UM create a distinct yet synergistic educational profile. Both programmes collectively contribute to innovation, capacity building, and resilience within the national pharmaceutical and healthcare landscape by diversifying academic environments and pedagogic approaches within the country.

Long-term Vision Toward 2030 and Beyond

Looking ahead, the programme is positioned to evolve dynamically in response to emerging scientific, clinical, and societal challenges. The coming decade is likely to see the development of specialized postgraduate pathways, deeper integration with biomedical engineering, microphysiologic systems, advanced materials science, and digital health technologies, fields in which the MF UM and FKKT UM already demonstrate strong interdisciplinary potential. Strengthening international visibility through ERASMUS+, joint degree programmes, and expanded research collaborations will further contribute to academic excellence and global relevance.

The programme scientific ambitions align closely with modern research frontiers in biotechnology, pharmacogenomics, advanced analytics, and drug delivery. These areas not only shape the future of pharmaceutical practice but also offer opportunities for cutting-edge translational research that can directly benefit the healthcare system and pharmaceutical industry in Slovenia. Importantly, the COVID-19

pandemic has underscored the vulnerabilities and systemic gaps within healthcare systems worldwide, highlighting the need for interdisciplinary problem-solving, rapid innovation, and flexible workforce deployment. The MF UM curriculum, rooted in biomedical science, enriched by industrial and technological insight, and deeply integrated with clinical practice, provides an effective framework for training professionals capable of responding to such complex, cross-sector challenges.

To sustain this long-term vision, ongoing investment in staff development, research infrastructure, and curricular flexibility will be essential. The rapid evolution of pharmaceutical sciences demands an educational model that remains responsive to new discoveries and technologies. The MF UM is strongly committed to monitoring professional, pedagogic, and scientific trends and continually updating the curriculum to ensure that graduates remain well-prepared for the evolving roles of pharmacists in healthcare, industry, and society at large. This continuous improvement mindset positions the programme not only as a timely response to present needs but as a forward-looking driver of innovation and resilience in the Slovenian healthcare and pharmaceutical ecosystem. Figure 5 summarizes the main specialities of the developed education model of pharmacy education at the MF UM.

CONCLUSION

The development of the Integrated Master's Programme in Pharmacy at the MF UM represents a major milestone for Slovenian higher education, healthcare, and industry. The programme is built upon a solid foundation of scientific, clinical, and industrial relevance that emerged from nearly 2 decades of conceptual exploration, four accreditation cycles, and extensive interdisciplinary collaboration. The successful accreditation of the programme in December 2024 reflects not only administrative competence but a deep alignment between academic vision and societal need. The "Maribor programme" establishes a second national centre for pharmacy education, one characterised by strong clinical integration, modern industrial and technological orientation, interdisciplinarity across faculties, and a commitment to regional development.

Looking ahead to the next decade, the programme is well-positioned to contribute to the pharmaceutical innovation ecosystem in Slovenia, address evolving workforce demands, and support the transformation of healthcare delivery. The establishment of the programme underscores the importance of strategic planning, evidencebased curricular design, and cross-sector partnership in advancing pharmaceutical education.

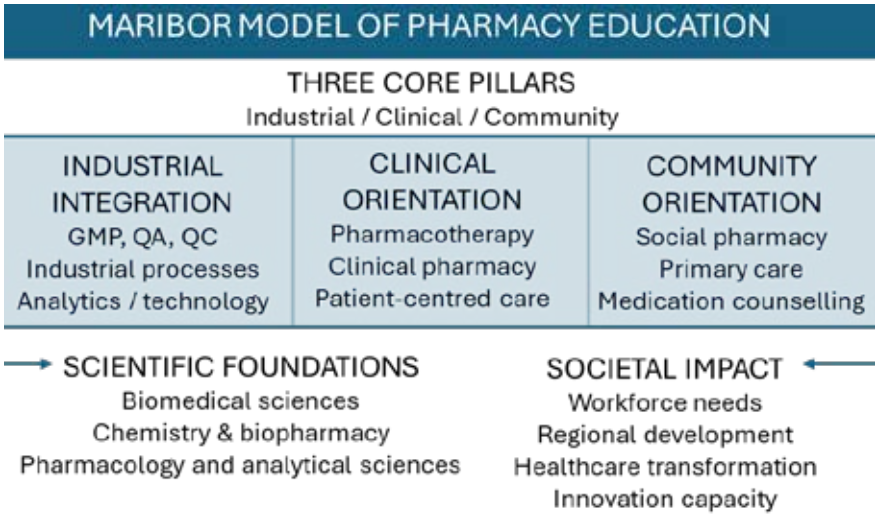

Figure 5. *The »Maribor Model« of the study programme.*

CONFLICT OF INTEREST

None.

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Uporaba botulinusnega toksina za zdravljenje glavobolov in bolečin obraza

Use of Botulinum Toxin for the Treatment of Headache and Facial Pain

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Izvleček

Botulinum toksin (BT) se pogosto uporablja v kozmetiki in klinični praksi. Ta pregled literature obravnava uporabo BT pri zdravljenju glavobolov in obraznih bolečin. Najbolj razširjena in dobro opisana ter odobrena uporaba je preprečevanje kronične migrene v odmerkih med 165 in 196 IE. Po naključnem odkritju je bila potrjena z več raziskavami RCT, predvsem s študijo PREEMPT. Po potrditvi je bila BT odobrena za uporabo pri zdravljenju kronične migrene. V skladu z veljavnimi smernicami se BT uporablja za preprečevanje kroničnih migren pri bolnikih, ki so odporni na druge oblike zdravljenja, npr. triptane ali nesteroidna protivnetna zdravila. Najpogosteje uporabljen protokol vbrizgavanja, ki je bil določen v študiji PREEMPT, priporoča 31–39 mest vbrizgavanja. Pri drugih primarnih

Abstract

Botulinum toxin (BT) is widely used in cosmetics and clinical practice. This literature review explores the applications of BT for the management of headaches and facial pain. The most widespread, well-reported, and ALIMS and EMA approved application for BT is in preventing chronic migraine at doses between 165–196 IU. After an incidental discovery, BT was validated through several RCTs, including most notably, the PREEMPT study. Following validation, BT was approved for use in treating chronic migraines. According to current guidelines, BT is used for the prevention of chronic migraines in patients who are resistant to other forms of therapy, such as triptans or NSAIDs. The most widely used BT injection protocol was established in

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glavobolih, kot so glavoboli tenzijskega tipa, je dokazov o učinkovitosti BT malo in so zmedeni. Pa vendar nekatere manjše študije poročajo, da je BT učinkovitejši od placeba in izboljša bolečino pri trigeminalnih avtonomnih glavobolih. BT se uporablja tudi pri kronični obrazni bolečini, predvsem trigeminalni nevralgiji, pri čemer so rezultati raziskav RCT spodbudni. Čeprav je glavni mehanizem delovanja BT zaviranje sproščanja acetilholina iz terminalnih holinergičnih živcev, je treba pojasniti še bolj specifične mehanizme lajšanja bolečine, zlasti pri migrenskih glavobolih. Po pregledu ustrezne literature je mogoče zaključiti, da je zdravljenje z BT varno, na splošno dobro prenašano in učinkovito.

the PREEMPT study and recommends 31–39 injection sites. In other primary headaches, such as tension-type headaches, there is little and confounded evidence of the efficacy of BT. However, some small-scale studies reported that BT outperformed placebo and improved pain in trigeminal autonomic headaches. BT is also used to treat chronic facial pain, most notably trigeminal neuralgias, with encouraging results in RCTs. While the primary mechanism of action of BT is the inhibition of acetylcholine release from terminal cholinergic nerves, more specific mechanisms of pain relief are yet to be elucidated, especially for migraine headaches. Our review of relevant published literature indicates that BT therapy is safe, generally well-tolerated and efficacious, and is a viable option for the management of certain primary headaches and chronic facial conditions.

INTRODUCTION

Botulinum toxin (BT) is a neurotoxic protein product of the bacillus Clostridium botulinum that induces muscle inactivity by releasing acetylcholine in cholinergic neurons (1). BT was discovered in the 14th century and first obtained in significant concentration in mid-20th century. BT was approved for the treatment of strabismus by the US Food and Drug Administration (FDA) in 1989 (2), which marked the beginning of its therapeutic use. The most common indications for the use of BT are focal and segmental dystonia (e.g., blepharospasm, cervical dystonia), muscle spasms, and in aesthetic medicine. BT is also indicated for hyperhidrosis, urinary incontinence, and migraines that have not responded to previous therapeutic approaches (3).

The use of BT is based on its inhibitory effects on the release of acetylcholine from presynaptic cholinergic neurons, which further affects the muscles or glands (3). BT is naturally found in seven serotypes (A–G), but only types A and B are used in therapy. Ona-, abo- and incobotulinumtoxinA and rimabotulinumtoxinB are four types of BT in wide clinical use and approved by the US FDA (3). Form A (Botulinum Toxin A – BTA) is the most widely used and most clinical applications are based on that variant (1). It is important to note that the forms and doses of different forms of BT are not equivalent.

Headache is one of the most common conditions in the general population (4), and about two-thirds of adults reported headaches in the previous year, most without further treatment or diagnosis (1). Migraine headaches are among the most disabling headaches and have a negative impact on quality of life (3). Headaches are classified according to the International Headache Society (5) system (ICHD-3 System) into primary (idiopathic) and secondary headaches. Primary headaches are divided into migraine, tension headaches, and trigeminal autonomic cephalgia (TAC), which includes the so-called cluster headache. Secondary headaches have different aetiologies, including cerebrovascular, traumatic, or headaches caused by neck pathologies and other maxillofacial structures, ear, larynx, sinus, and nose (5).

After the accidental discovery that BT has a positive effect on reducing headaches, its use in pain treatment was investigated (6). Since 2010, BT has been used

for the prevention of chronic migraines that do not respond to other forms of therapy (3).
BT is also used for chronic facial pain caused by trigeminal neuralgia, temporomandibular joint, and dental pain (3). The use of BT to treat headaches and facial pain has increased since the discoveries made in 2010, with multiple clinical trials, both approved and off-label uses, reported in the literature (6, 7). The mechanisms of action of BT in pain treatment are not fully understood, although recent discoveries provide some explanations (6). This report reviewed the relevant literature and details the therapeutic use of BT in headaches and facial pain.

PHARMACOLOGY AND MECHANISMS OF ACTION OF BOTULINUM TOXIN

BT is the product of the anaerobic Gram-positive bacillus, *Clostridium botulinum*, with seven serotypes that each have a distinct molecular structure. Serotypes A and B are used in the pharmaceutical form. The neurotoxin complex is composed of a toxin (molecular weight of 150 kDa) and a non-toxic protein complex that protects the toxin from deactivation by deactivating factors. The toxin itself (Figure 1)

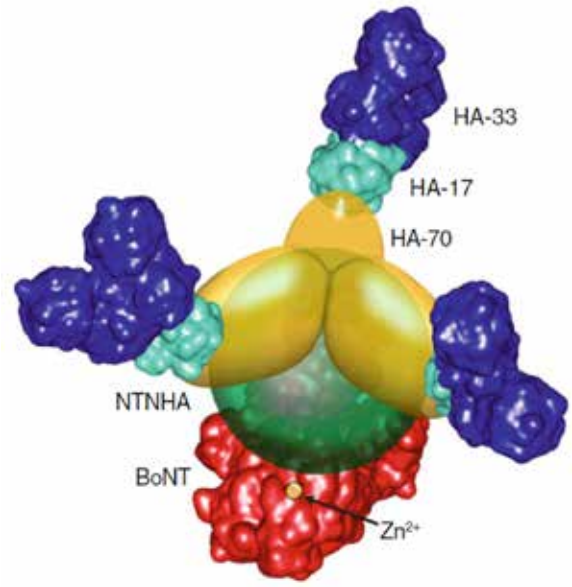

Figure 1. *Molecular structure of BT, based on Jabbari. (3)*

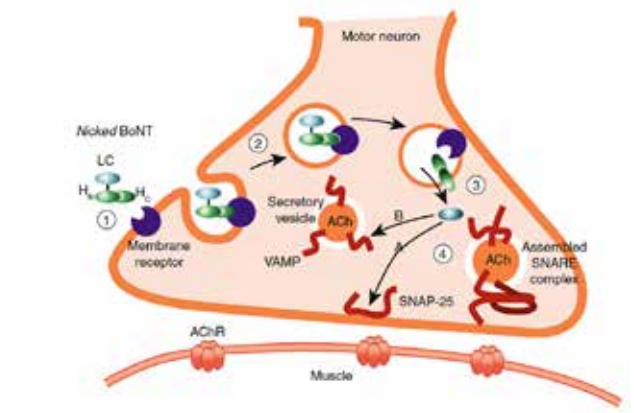

Figure 2. *Synaptic action of BT on the neuromotor plate (schematic), based on Jabbari. (3)*

consists of a heavy chain (HC) of 100 kDa (kilodalton) and a light chain (LC) of 50 kDa bound by a single disulfide bond. The HC contains the protein binding and translocation domains, while the LC is a catalytic domain (3).
Within one minute of intramuscular injection, the toxin dissociates from the protein complex and activation occurs. Serotypes A and B have different membrane receptors, and the toxin is released into the endosome, where the disulphide bond and the HC-LC complex are dissasociated. Then, the HC domain opens a channel in the cell membrane and translocates the LC domain to the cytosol. The LC contains the chemical properties of the zinc-motif proteases and catalyses trans-membrane SNARE complexes present in the presynaptic space. Deactivation of the SNARE complex (Figure 2) causes vesicular fusion and neurotransmitter (mainly acetylcholine) release. After several hours of neurotransmitter release,skeletal muscle weakening becomes observable; however, the clinical paralysis of the muscle becomes evident after seven days. The effect lasts 3 to 6 months. In addition to its action on the neuro-muscular surface, BT binds neurotransmitters on the autonomic ganglia, post-ganglionic sympathetic nerve impulses, and post-ganglionic parasympathetic nerve impulses (8).
The effect of BTA on headaches can be observed on four levels. At the level of axonal transport and transcytosis, model studies suggest the effect of BTA

on anterograde and retrograde axonal transport from nerve terminals to adjacent neurons and glial cells. Injections into M. occipitalis, near N. Occipitalis minor and major, indirectly affect the nociception of the cerebral sheaths. At the neurotransmitter inhibition level, BTA inhibits the release of other neurotransmitters associated with migraine, such as CGRP (Calcitonin gene-related peptide, a migraine mediator), supplement P and glutamate, as well as serotonin, GABA (gamma-aminobutyric acid), norepinephrine, dopamine, and glycine. At the level of neuromodulation, BTA inhibits the expression of several nociceptive receptors (TRPV1, TRPA1, PRX3, TRPM8 and GABA-A), with an agonistic effect on μ-opioid receptors. On the fourth level, BTA modulates cytokines via the anti-inflammatory inhibition of pro-nociceptive interleukins and the stimulation of anti-nociceptive interleukins, which play a role in the inflammatory component of migraine pathophysiology (1).

Initial assumptions that the analgesic effects of BT rest on myorelaxation, leading to the decompression of local blood vessels, are changing in the light of new research. The analgesic action of BT is also considered to be due to its direct action on sodium channels and its ability to reduce the pain-mediating effect of Substance P. Migraine studies have shown that BT acts on the pain mediators Substance P, calcitonin-generated peptide (CGRP), and glutamate, which are essential for the development of migraine (9).

The pharmaceutical form of BT depends on the serotype and the manufacturer. BOTOX® by Allergan will be described here according to the Summary of Medicinal Product Characteristics of the Agency for Medicinal Products and Medical Devices (ALIMS). The medicinal product is available in doses of 50, 100 and 200 units per 0.1 mL. The medicinal product is a white powder that is hardly noticeable at the bottom of the bottle. The reconstituted dilution is clear or slightly yellow. The drug is reconstituted with sterile saline, without preservatives. ALIMS recommends using a bottle with 100 units for easier reconstitution (10). Information on administration in specific cases is provided in the following text.

When used correctly, the adverse effects of BT are rare and minimal. Such adverse effects are related to the injection site itself and to the rare occurrence of systemic adverse effects such as muscle weakness or difficulty swallowing. The text below will provide information on specific adverse effects for different indications. However, BT is considered a drug that is generally well tolerated, and it is significant in treating chronic conditions such as migraines (11).

USE IN HEADACHES

Migraine

Migraines; i.e. migrainous headaches, are among the most painful conditions, with significant representation in the world population (12). Migraines are more prevalent in females at a female-to-male ratio of 2:1 and are most often seen in individuals between 35 and 45 years of age (12). Migraines are presented as frontotemporal headaches with lateralisation (1). The main categorisation of migraines is based on the presence of aura; i.e. sensory changes. Twenty percent of migraines are presented as classic migraines with aura, and the rest are migraines without aura (5). The ICHD-3 system further defines the diagnostic parameters of migraine as episodic or chronic. Chronic migraines are considered migraines where the headache is present for 15 days per month, with migrainous characteristics present for eight of the 15 days (5). Other migraines are considered episodic. The most severe forms of migraine are accompanied by significant changes in vital functions (blood pressure, heart rate, respiratory rate) that must be monitored (13). Although less frequent, chronic migraines are associated with significant health and psycho-social-economic consequences and a significantly lower quality of life (1).

Migraine treatment is carried out in three phases: preventive measures, such as lifestyle changes and the elimination of the triggers; prophylactic treatment; and acute treatment (1). During the acute treatment of migraines, NSAIDs (non-steroid anti-inflammatory drugs) and triptans are used in treating minor cases (3). The use of triptans is limited by cardiovascular comorbidities (3). Topiramate, beta-blockers, histamine and BT can used for prophylaxis.

Newer drugs include CGRP-specific monoclonal antibodies, which act directly on 5-HT receptors in the trigeminovascular nerves, preventing the onset of migraine attacks (14).

Clinical studies on BT for migraines

The potential efficacy of BT for the treatment of migraine was accidentally revealed by patients receiving BTA injections for the aesthetic treatment of facial wrinkles and who experienced the coincidental relief of migraines. Such accidental findings initiated the first open-label clinical trial of BTX-A on 106 patients in 2000. As a key result of that trial, out of 77 patients with the most significant diagnostic criteria for migraine, 36 reported complete elimination of headaches. Although promising, the results of that study were limited by the study design itself, the selection of the study population, and the random categorisation of migraine, as well as by the use of inconsistent methods for measuring improved responses (15). The results of the trial were also used as the basis for the first double-blind, randomised, placebo-controlled clinical trial of BTA.

The use of standardised doses (25 and 75 IU) and standardised pericranial injection sites points to the validity of the aforementioned clinical trial. Patients were assigned to two treatment groups, numbers 2 and 3, which received doses of 25 IU and 75 IU, respectively, and a control group (number 1, placebo). Patients in group 2 reported a reduction in the number of migraines over a month and a reduction in the intensity of migraines, while such improvements were not observed in group 3. No significant adverse events were observed (15).

The two PREEMPT studies on the use of BTA for migraines have been the most important clinical studies confirming the efficacy of BTA (ona-BTA) as a prophylaxis for chronic migraines. However, those studies had limitations. The PREEMPT 1 study was set up as a 24-week randomised, double-blind clinical trial, followed by a 32-week open-label phase, conducted on males and females aged 18–65 years with chronic migraine identified according to the criteria of the then-current ICHD2 standard (16). Ona-BTA was injected in 31 sites on the face and neck according to the protocol, which has since been called the PREEMPT injection protocol. The primary objective of the first PREEMPT study was the mean improvement in the number of headache episodes. That objective was not achieved, while the secondary objectives were achieved. Therefore, the first PREEMPT study was characterised by a high degree of placebo effect. The PREEMPT 2 study aimed to establish a mean improvement in the monthly frequency of headaches and was successful. The pooled analysis showed a statistically significant ($p \leq 0.0027$) advantage of OntobotulinumtoxinA over placebo for the entire 24 weeks of treatment, in terms of the mean improvement in relation to the initial number of headache episodes per month, headache episodes described as moderate, headache episodes, and migraine episodes (16). Additional analyses showed that while the frequency of headaches had not significantly changed in patients with headaches, the severity of headaches did change. Thus, that clinical study showed that patients who received BTA therapy experienced less headache pain measured by the Headache Impact Test 6 (HIT-6) and an improvement in the Migraine-Specific Quality of Life (MSQL) score. Of the adverse events, 4% of patients in the treatment group reported neck pain, 2% reported musculoskeletal pain, and 1% experienced blurred vision (17). Although the PREEMPT studies demonstrated the efficacy of BTA for migraine treatment, the placebo effect was significant and raises questions about the validity of the data (16). The PREEMPT studies, as well as the multinational, open-label COMPEL study, which investigated the clinical benefits of long-term BTA therapy for chronic migraine for two years, confirmed the results of PREEMPT and provided sufficient authoritative evidence for the broader use of BTA as migraine prophylaxis and the foundation for regulatory BTA approvals by the world's major regulatory authorities (16), such as the FDA (2010) and the European Medicines Agency (EMA) in 2010. The FDA established chronic migraine prophylaxis (>15 headache days per month) as an indication for the use of Ont-BTA. Approval in the EU was based on inter-institutional recognition via the Irish regulator.

Indications, however, may differ slightly between member states or may be based on preventing chronic migraines (16). The Serbian ALIMS approved the use of Botox® produced by Allergan Pharmaceuticals in 2021 for multiple indications, of which the most significant was the "alleviation of symptoms in adults with chronic migraine, who meet the criteria for diagnosis (≥15 headache days per month, of which at least eight days with migraine headaches) and patients who do not respond adequately or are intolerant to migraine prophylaxis drugs" (10).

Therapeutic application, protocols, tolerance and therapy efficacy

Several therapeutic guides, including the one by the European Headache Federation, recommend BTA to prevent chronic migraines as one of the two recognised and approved methods, especially in cases where patients do not respond to other therapies or do not tolerate other forms of treatment. The European Headache Federation considers that the evidence for BTA use for chronic migraine is high and that the treatment is effective and well tolerated by patients (18). The same guideline recommends BTA use in patients in whom at least two to three migraine prophylaxis regimens have not been effective, excluding contraindications due to comorbidities. The guideline illustrates the overuse of drugs, especially analgesics in migraine patients, and recommends detoxification before BTA use. The guideline recommends using headache calendars to evaluate the response to continued treatment by comparing the four weeks before and four weeks after each treatment cycle. Discontinuation of the treatment is recommended in patients with less than ten headache days over three months. Re-evaluation is recommended 4–5 months after the discontinuation of therapy (18).

The main injection techniques are categorised as fixed injection sites to track the number and location of injections and "follow-the-pain" techniques that use asymmetric injection sites that are oriented towards the pain sites reported by the patient. Combined techniques use fixed injection sites in the frontal regions, but use supplementary injections for pain monitoring and are characterised by a higher BTA dose. Of the established injection protocols, the most widely used in the literature is the protocol of fixed injection sites defined in the PREEMPT study – the PREEMPT injection protocol (9). This protocol is also recommended by the European Headache Federation (18).

The PREEMPT protocol recommends a dose of 155–195 U of Ont-BTA as intramuscular injections in 0.1 mL (5 IU) into 31 to 39 sites in muscular regions (Figure 3): M. corrugator, 10U at two sites; M. procerus, 5U per 1 site; M. frontalis, 20U per 4 sites; M. temporalis, 40–50U per 8–10 sites; M. occipitalis, 30–40U per 6 to 8 sites; cervical paraspinal muscle group, 20 U per 4 sites, and M. trapezius, 30–50U per 6–10 sites. All muscles should be bilaterally injected, except for M. procerus, which should be injected only in a single central place. The recommended treatment period is every 12 weeks (16).

Cilinical stuidies of migraine consider higher doses more effective and indicated for patients who do not improve with lower doses. Additional doses over 195 IU are indicated in protocols for pain management, where the use of conventional injection sites is also recommended, except for the temporal muscle, where two additional injection sites are recommended. It is recommended to avoid additional injection sites in the M. trapezius (19).

Other injection protocols, such as the one developed at Yale University, with 23 injections and a total dose of 165 IU, emphasize the injections into the temporal muscle but exclude the M. trapezius completely. There are discrepancies between authors regarding whether injections into the temporal lobe result in a greater number of unwanted events, such as muscle weakness (3). The choice of injection protocol and whether to increase the dose above that prescribed by the PREEMPT protocol is individual and depends on patient factors, such as the anatomical characteristics of the patient, the patient's tolerance, and the occurrence of adverse effects such as post-procedure main (9). Studies suggest that the therapeutic results are improved when using an experienced practitioner who has a good understanding of the functional anatomy

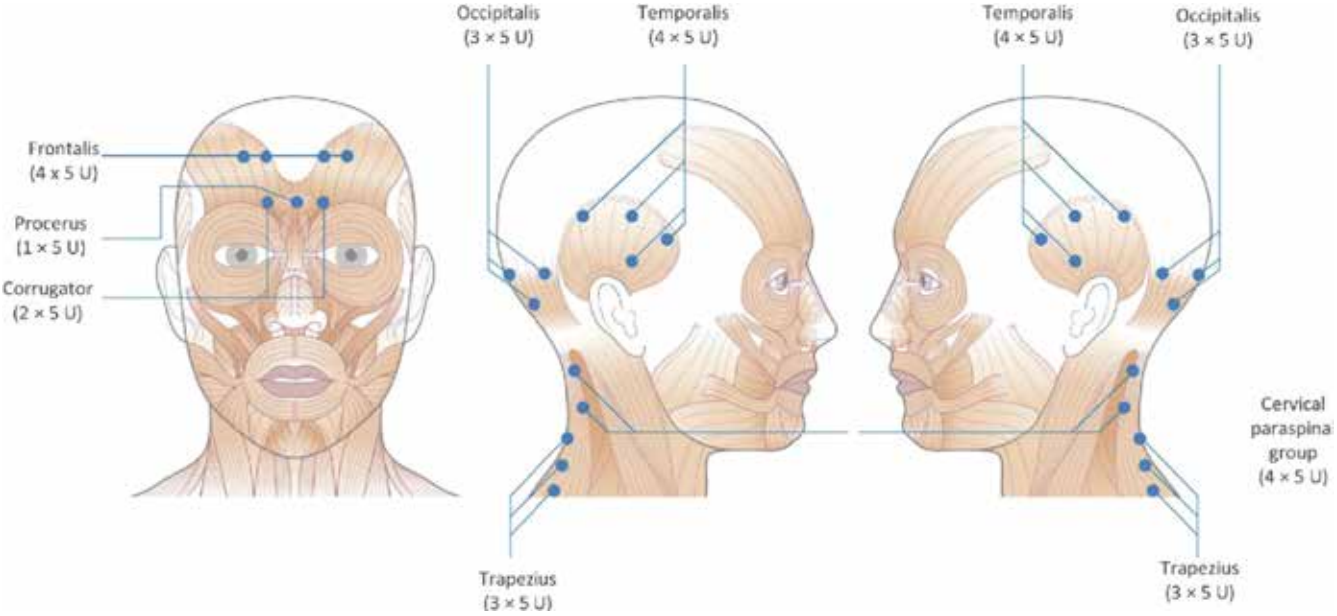

Figure 3. *Injection sites according to the PREEMPT protocol, by Tassorelli C, Sances G, Avenali M, De Icco R, Martinelli D, Bitetto V, et al. BT for chronic migraine: clinical trials and technical aspects. Toxicon. 2018; 147: 111–5.*

of the injected muscle, who has conducted a detailed examination of the patient before the injection, who monitors their side effects (pain, discomfort, muscle weakness) during the interventions, and who employs correct injection techniques (6).

Patients tolerate BTA better than other oral migraine prophylaxis drugs (6). Recent publications classified patients with chronic migraines according to their response to BT therapy, the number of days of headache, and their response to relevant treatment questionnaires (Migraine-Specific Quality of Life Questionnaire), as follows: (1) Excellent response: patients with >75% reduction in headache and who were recommended to increase the dose to 195U using a single injection protocol for three months before reducing the interval to 4 months during the following year and to cease treatment at the end of two years with the same response. (2) Good response: Patients with a 50–70% reduction in headache days, with newer protocols suggesting adjunctive new therapies. (3) Poor response: Patients with a 30–50% headache reduction with either a combination of oral prophylactics (triptans) and BT treatment being recommended for one year, or a change in therapeutic approach in favour of newer therapies (antibodies targeting CGGRP). (4) Patients without a significant response: those with <30% reduction in headache after the second treatment and the addition of oral therapy and discontinuation of BT therapy in favour of other therapeutic options (17).

Several predictive factors determine the success of BT therapy. Patients who respond better to the treatment have more implosive, but not explosive, headaches. The same applies to ocularly-located headaches and early therapy within 12 months after the diagnosis of chronic migraine (9).

BT use is best described in adults, has received regulatory approval for use in adults, and is only indicated for chronic migraines in adults. Off-label use in episodic migraines is described in the literature, but such use is sporadic, and there is insufficient data to justify extending the indication. Off-label use of BT for episodic migraines as an alternative therapy in cases of an inadequate response to other therapeutic options, however, is not unjustified, and such therapeutic

applications should follow the existing therapeutic protocols for chronic migraines. (6). Data and studies on the off-label use of BT for paediatric migraines are scarce, and there is insufficient evidence to justify such use. Nonetheless, randomised clinical trials and paediatric investigative plans for the use of BT in paediatric migraines are in progress. Such studies are being implemented to improve the knowledge and treatment of paediatric chronic migraine (20).

Tension headaches

Tension-type headaches are the most common primary headache. Symptoms of tension-type headaches include dull and irritating pain, sore head muscles and neckaches. Tension headaches are generally less intense than migraines, but they can have a devastating effect on the quality of life. Similar to migraines, tension headaches can be episodic and chronic. Chronic tension headaches have a similar incidence in about 2% of the population (5). Tension headaches are more common in females, as well as in people with a higher level of education, while the triggers include stress and other psychological factors (3). Recent experimental studies indicate that psychological conditions such as anxiety, depression and stress play a significant role in the sensitisation of sensory and neurological pathways, and disturbed nociception, possibly at the level of cholinergic, serotonergic, and inflammatory mechanisms (21). Episodic tension headaches are treated to NSAIDs and ancillary therapeutic methods such as lifestyle modification or psychotherapy. Tricyclic antidepressants and SSRI antidepressants are also used for chronic tension headaches.

In his textbook on the use of Botox for therapy (3), Jabbari details six prospective double-blind studies, of which three are classified as first-class studies according to the American classification. Similar to studies investigating migraines, the studies detailed in the textbook by Jabbari focused on chronic tension-type headaches and doses of BT between 50 and 250 IU. BT did not cause a significant improvement over placebo in any of the studies (3). Generally, studies aiming to establish the effectiveness of BT in treating chronic headaches are inconsistent. Studies on the use of BT in tension headaches are inconsistent, and while some exhibit limited study designs, overall we can observe significant differences in the doses and injection protocols used, making inferences difficult to make. Some studies have had contradictory results, and in studies with small cohorts, BT treatment caused a decrease in the average number of days of headaches in patients and a reduced need for other drug treatments (9, 22, 23). A recent meta-analysis investigating 12 clinical studies for the use of BTA in the prevention of tension headaches found no evidence that BTA injections were superior to placebo in treating severe headaches (21).

Trigeminal autonomic headaches

Trigeminal autonomic headaches are characterised by symptoms such as unilateral headache with prominent ipsilateral cranial autonomic features, lacrimation, conjunctival injections or nasal symptoms. Unilateral headache is a key characteristic of these conditions. They qualify as primary headaches, and the most prominent trigeminal autonomic headache is a cluster headache, while paroxysmal hemicrania, SUNCT (short-lasting unilateral neuralgiform headache attacks with conjunctival injection and tearing), and SUNA (short-lasting unilateral neuralgiform headache attacks with cranial autonomic symptoms) headaches also fall under this category (5, 24). In pathophysiological terms, trigeminal autonomic headaches are characterised by the facilitation of the trigeminal autonomic reflex. The posterior hypothalamic region is currently considered to be crucial for the pathogenesis of trigeminal autonomic headaches since the activation of the region ipsilateral to the affected side is the focus of neuroradiological studies in patients with trigeminal autonomic headaches (24, 25).

A relatively recent systematic review of the literature (24) identified three essential studies from 2007, 2016, and 2018 that investigated the uptake of BT in treating cluster headaches. All three studies were prospective, open-label, and without a control group. Two studies used the PREEMPT injection protocol, while one used injections directly into Meckel's ganglion (Ganglion pterygopalatinum). (26). A specialised applicator was required in a trial

where BTA was administered directly to the G. pterygopalatinum as a block due to inadequate drug diffusion. (27). All three studies showed significant improvements in headache frequencies from as early as the first week after treatment, with a duration of up to 6 months. In a study where the ganglion was targeted directly under anaesthesia, the authors noted significant adverse effects in the patient, including significant bleeding, accommodative weakness of the ipsilateral eye and difficulty chewing. However, studies that used PREEMPT do not show significant adverse events thus far. Although promising, those studies have limitations ranging from a small number of participants to the lack of control groups. Future randomised, placebo-controlled studies may confirm the findings of the studies on BT use in cluster headaches. Considering the pathophysiology of cluster headaches and other trigeminal headaches, it is reasonable to assume that BT could act on the mechanisms responsible for their occurrence, both by inhibiting neurotransmitter release and pain initiation, and by reducing the peripheral sensitisation of nociceptive sensory nerve fibres. Although the studies focused only on chronic cluster headaches, it is assumed that positive therapeutic effects on episodic cluster headaches and other trigeminal headaches are possible (26).

Secondary headaches

Secondary headaches occur at close time intervals to other conditions that cause headaches, where the causal links between primary pathology and secondary headaches are well-known and established. The treatment of secondary headaches aims to treat the primary conditions that trigger the headache. A 2011 retrospective study (28) analysed previously published clinical studies investigating different types of secondary headaches, including primarily neurogenic headaches, and concluded that there is insufficient evidence to support the use of BT in treating secondary headaches. However, two small studies demonstrated that BT can be beneficial for the treatment of cervical dystonia. Following the studies, the FDA approved BT to treat cervical dystonia, a condition that may trigger secondary headaches. In cervical dystonia, injection is administered to the affected muscles, with or without electromyography or ultrasound guidance (29).

A notable, small, randomised clinical study of patients with PTSD indicated that the use of BT following the PREEMPT protocol significantly reduced the frequency of headaches and the level of pain in post-traumatic headaches in trauma veterans (30).

USE IN FACIAL PAIN

Successful facial pain therapy represents a significant challenge in modern pain medicine. Most facial pain is of neuropathic origin, and the use of BT for the treatment of this type of pain is based on the latest research. This section focuses on trigeminal neuralgia, temporomandibular pain, and conditions with sufficient relevant literature on the use of BT. It should be noted that studies on other maxillofacial conditions aside from bruxism are not included in this literature review.

Trigeminal neuralgia

Trigeminal neuralgia is a rare condition and causes one of the most excruciating pain in humans, characterised by unilateral shock-like facial pain. Although the exact prevalence of trigeminal neuralgia is unknown, females are more often affected than males. Dental problems are a frequent initial diagnosis of trigeminal neuralgia, especially when the lower trigeminal branches are involved. Neuralgia can affect any of the trigeminal nerves; however, the ophthalmic and maxillary branches are most commonly affected. The pain is short-lived; however, it has pronounced symptoms, and attacks of pain can occur multiple times per day and can be triggered by a gentle touch. Current empirical evidence suggests that the pathogenesis of trigeminal neuralgia is based on the vascular compression of the trigeminal nerve root (31). The pharmacological approach to treat trigeminal neuralgia is based on antiepileptic drugs such as carbamazepine, oxcarbazepine, or GABA-peptide, which block pain mediators, and drugs that act on GABA receptors such as baclofen, which boost inhibitory mechanisms. The simplest and most

common tool for assessing pain and the effectiveness of therapy is the VAS pain scale (32). Surgical treatment is indicated for patients who cannot manage their condition with medication alone (3).

A 2001 study, which included patients with chronic facial pain, showed that in 8 out of 11 patients, BT treatment resulted in a positive response, including reduced pain episodes (33). In his textbook, Jabbari described eight clinical studies of the efficacy of BT in treating trigeminal neuralgia, focusing on two prospective blinded studies. In the first 2012 study, 42 patients with trigeminal neuralgia participated in a 13-week randomised, parallel-designed, double-blind, placebo-controlled study. BTA, dissolved in 1 cc of saline, was injected with a 16 mm needle between the epidermis and dermis, or submucosally in the affected area, with a total concentration of 25–27 IU BTA. Patients in the treatment group showed a statistically significant ($p < 0.001$) change in the frequency and intensity of pain (VAS score). In another 2012 single-blinded study, patients were administered 40–60 IU BTA to 8–12 affected areas. A statistically significant ($p = 0.0001$) reduction in VAS score was observed 12 weeks after treatment, as was a significant improvement in quality of life and a reduced need for analgesics. (3). A 2020 meta-analysis (34) concluded that most studies on the use of BTA in trigeminal neuralgia have statistically significant and valid results supporting the use of BTA due to its positive effect on the frequency and intensity of pain, and the overall improvement in quality of life. BTA can be used alone or as an adjuvant therapy. Serious side effects include transient facial asymmetry and oedema, while mild side effects include injection site irritation and haematomas (34).

Temporomandibular joint disorders

Temporomandibular disorders (TMJ disorders) are a group of conditions related to the pathological processes of the jaw and the masticatory muscle, and can be arthrogenic (originating in the join) or myogenic (originating in the muscle). Arthrogenic prolapses are caused by inflammatory pathologies of the temporomandibular joint, while myogenic TMJ disorders are caused by pathologies of the masticatory muscles: M. masseter, M. temporalis, and M. pterygoideus lateralis. Pain is a defining characteristic of those conditions and can be either localised at the temporomandibular joint or in the masseter muscles. Conservative treatment methods are non-invasive, such as massage, warm compresses, and physical therapy. Pharmacological treatment is performed with NSAIDs, myorelaxants, tricyclic antidepressants, and antiepileptic analgesics. Surgical intervention is considered the last option for treatment (3).

A2018 study (35) used retrospective methods to establish the efficacy of BTA in treating temporomandibular joint disorders. The injection protocol used for those conditions was as follows: 100 U of BTA was injected into ten sites, three along the masseter and two along the temporalis. If necessary, the dose was increased to 150 IU or decreased to 50 IU in patients experiencing relief. The injection zones are called "trigger" zones because of the electromyogram activity, which is deemed preferable regarding the anatomical identification of the injection zones requiring more experience in administration. The study(35) exhibited encouraging results, showing that BT injections had significantly improved symptoms in 80% of patients. It was concluded that BT can be used as a line of therapy in cases where other conservative, non-invasive methods do not have a significant effect. (35).

A 2019 study focusing exclusively on myogenic conditions resulted in a positive effect in more than half of the patients and concluded that BT has a place in treating that subgroup of temporomandibular conditions, alongside other treatment options (36). However, the limitation of the discussed studies lie in a small cohort, and further studies are necessary to demonstrate and validate the use of BT in temporomandibular disorders. Expertise in BT administration is also a significant predictor of treatment success (37).

CONCLUSIONS

By reviewing the existing literature, this series attempted to detail the use of BT in treating headaches and facial pain. The largest volume of studies and the most convincing evidence has been in migraine prophylaxis. Twelve years after regulatory approval and almost twenty years since its first use, BT is effective in treating migraines that do not respond to other treatments, and there is sufficient evidence to support its clinical use. Although some authors emphasise the financial burden of using BT for migraine and question the effectiveness of the drug, there are significant data highlighting the improvements in the quality of life of patients undergoing such treatment to justify its use. The totality of evidence also suggests patients' good adherence to the treatment and minimal reporting of adverse events, which speaks in favourability of this therapy.

There is a consensus in the literature regarding significant experience in treating BT, which prevents adverse effects in multiple ways. Advances in the development of modern therapies, particularly monoclonal antibodies, as well as an understanding of migraine pathogenesis, have contributed to the improved treatment of migraine; however, there are indications that BT therapy will continue to be an essential adjuvant given its good tolerance and relative non-invasiveness.

Conversely, for other primary headaches, particularly tension headaches, there is no scientific consensus on the use of BT, and the total number of clinical studies performed to date shows no progress in the use of this therapy. BT has its place in the treatment of pathologies causing facial pain, especially temporomandibular disorders, where further clinical studies will confirm or refute the justification for the use of BT.

Although not definitive, this literature review has advanced the picture of BT use in treating headache and facial pain. Further studies are needed to support BT use for pain treatment, focusing on understanding its mechanisms of action for pain reduction.

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Sindrom razdražljivega črevesja: ali je učinkovita rešitev še vedno tako nedosegljiva?

Irritable Bowel Syndrome: Is an Effective Solution Still Elusive?

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Izvleček

Sindrom razdražljivega črevesja (SRČ) spada med motnje v interakciji med črevesjem in možgani ter zaradi svoje kompleksne patofiziologije predstavlja diagnostični in terapevtski izziv tako za bolnike kot tudi zdravstvene strokovnjake. K obravnavi bolnika je treba pristopiti postopno – z natančno klinično oceno, izključitvijo alarmnih simptomov in diferencialno diagnostično obravnavo drugih obolenj, kot so kronična vnetna črevesna bolezen, rak debelega črevesa in danke, celiakija, divertikulitis, malabsorbcija ogljikovih hidratov, kronični pankreatitis, nevroendokrini tumorji, hipertiroidizem, bakterijska razrast tankega črevesja in gastrointestinalne okužbe, ki jih je mogoče zdraviti bolj specifično ali uspešnejše. Po potrjeni diagnozi je terapevtski pristop usmerjen v ublažitev glavnih simptomov,

Abstract

Irritable bowel syndrome (IBS) is classified as a disorder of the gut-brain interaction. IBS presents an ongoing challenge for patients and healthcare professionals due to the complexity of the underlying pathophysiology. It is important to approach the patient with IBS in a stepwise manner, beginning with the exclusion of alarming symptoms and other conditions, such as inflammatory bowel disease, colorectal cancer, celiac disease, diverticulitis, carbohydrate malabsorption or maldigestion, chronic pancreatitis, neuroendocrine tumours, hyperthyroidism, small intestinal bacterial overgrowth, bile acid malabsorption or idiopathic bile acid diarrhoea, and gastrointestinal infections, which often have more straightforward or effective treatment. When

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kot so abdominalna bolečina, napihnjenost ter spremenjena frekvenca in konsistenca blata.
Celostno vodenje bolnika vključuje vzpostavitev trdnega terapevtskega odnosa med zdravnikom in bolnikom, spremembo življenjskega sloga (vključno s telesno vadbo in prehrano), oceno črevesne mikrobiote, uporabo probiotikov, individualno prilagojeno farmakološko terapijo ter psihološke pristope, kot sta psihoterapija in obvladovanje stresa. Kljub mnogim, že dostopnim terapevtskim možnostim, ostaja iskanje optimalne in dolgoročno učinkovite rešitve za SRČ še vedno aktualen izziv.

the diagnosis of IBS is established, the focus is on therapy aimed at alleviating symptoms, such as pain, bloating, and altered bowel habits. Management of IBS includes a strong patient-physician relationship, lifestyle modifications (physical activity and dietary changes), analysing the gut microbiota, using probiotics, pharmacologic therapy, and psychological support, including psychotherapy and stress modulation.

INTRODUCTION

Irritable bowel syndrome (IBS) is classified as a functional gastrointestinal disorder. Notably, the pathophysiology underlying IBS is poorly understood and the treatment outcomes are frequently suboptimal. The most recent Rome IV diagnostic criteria have introduced a revised classification, in which the functional gastrointestinal disorders have been renamed as disorders of the gut–brain interaction. This terminology emphasizes the multidimensional nature of IBS and the necessity of a multidisciplinary approach to management (1).

Epidemiologic data from 2022 estimate that IBS affects between 10% and 23% of the global population (2). IBS affects all age groups with approximately one-half of patients < 35 years of age. Women are disproportionately affected by IBS with higher prevalence rates reported in Asia and Europe. Spontaneous remission of symptoms occurs in 12%–38% of patients. Physicians at all levels of healthcare from general practitioners in primary care to specialists in secondary and tertiary settings increasingly encounter individuals presenting with IBS-related symptoms. Clinical management of IBS is often complex and resource-intensive, involving multiple referrals, imaging studies, and invasive diagnostic procedures. Despite these efforts, therapeutic outcomes for IBS are frequently unsatisfactory (3, 4).

The etiopathogenesis of IBS has not been completely elucidated. Current evidence suggests that IBS most likely arises from an interplay of psychological and environmental factors, which trigger alterations along the gut–brain axis (4).

DEFINITION OF IBS

The Rome IV criteria (2016) remain the standard diagnostic framework for the initial clinical evaluation of patients suspected to have IBS (5). According to these criteria, diagnosing IBS requires recurrent abdominal pain, occurring at least 1 d/w over the past 3 months, accompanied by at least two of the following features:

- improvement of pain after defecation;
- change in stool frequency; and
- change in stool form or consistency (5).

Based on stool characteristics, IBS is further classified into subtypes:

- IBS with predominant constipation (IBS-C), > 25% of bowel movements with Bristol stool form scale (BSFS) types 1 or 2 and < 25% with BSFS types 6 or 7;
- IBS with predominant diarrhoea (IBS-D), > 25% of bowel movements with BSFS types 6 or 7 and < 25% with BSFS types 1 or 2;
- IBS with mixed bowel habits (IBS-M), > 25% of

bowel movements with BSFS types 1 or 2 and > 25% with BSFS types 6 or 7; and
- unclassified IBS (IBS-U), patients who fulfil the diagnostic criteria for IBS but do not meet criteria for any of the defined subtypes (4, 5).

DIAGNOSTIC CONSIDERATIONS AND EXCLUSION OF ORGANIC DISEASE

Urgent conditions and organic causes of symptoms must be excluded before confirming the diagnosis of IBS based on the Rome IV criteria. A thorough medical history is essential to characterize the pattern of abdominal pain, the relationship to defecation, and identify potential alarm features, such as haematochezia or melena, unintentional weight loss (> 10% over the previous 6 months), iron deficiency anaemia, nocturnal symptoms, onset after 50 years of age, or a family history of colorectal cancer or inflammatory bowel disease. The presence of any alarm feature necessitates further diagnostic evaluation, referral to a gastroenterologist, and/or endoscopic investigation (5, 6).

In addition, disorders that mimic IBS should be systematically excluded. In addition, evaluating a patient with suspected IBS should include the following: a review of ongoing and newly introduced medications; serologic testing for celiac disease; assessment for bile acid diarrhoea following cholecystectomy; stool cultures to exclude infectious causes of diarrhoea; and baseline laboratory testing. Only after these possibilities are excluded should the Rome IV criteria be applied to establish a diagnosis of IBS (5, 6).

Routine testing for C-reactive protein, food allergies, carbohydrate malabsorption, or faecal calprotectin is not recommended during the initial diagnostic work-up for IBS (7). Nevertheless, faecal calprotectin (FC) remains an important biomarker of intestinal inflammation in inflammatory bowel disease (IBD) but the results must be interpreted in the context of the overall clinical presentation because levels may also be elevated in other gastrointestinal disorders. Markedly elevated FC values (> 250 µg/g) effectively exclude IBS as the sole underlying pathologic disorder (8).

In cases of refractory symptoms or poor treatment response, additional differential diagnoses should be considered, including defecatory or motility disorders with altered intestinal transit times, carbohydrate malabsorption or maldigestion (e.g., lactose or fructose intolerance), chronic pancreatitis, neuroendocrine tumours, hyperthyroidism, small intestinal bacterial overgrowth (SIBO), bile acid malabsorption, and idiopathic bile acid diarrhoea. The latter disorder is characterized by an increased luminal bile acid concentration, particularly after cholecystectomy, leading to enhanced secretion, permeability, and motility (4,7). In such cases, bile acid sequestrants, such as cholestyramine, may provide significant symptomatic relief (9).

MANAGEMENT STRATEGIES

IBS results from disturbances in bidirectional communication between the brain and the gut. This gut–brain interaction is influenced by multiple factors, including genetics, personality traits, individual stress reactivity, mucosal inflammation, alterations of the intestinal microbiome, and sequelae of bacterial, viral, or parasitic infections (9). Among these, stress reactivity, mucosal inflammation, microbiome composition, and post infectious changes are now considered modifiable, allowing for symptomatic relief and improvement in patients' health-related quality of life.

Patient–physician relationship and lifestyle modification

Successful therapeutic management begins with the establishment of a respectful and empathetic patient–physician relationship. Many patients hold misconceptions and unrealistic expectations regarding their condition and some healthcare providers still believe IBS is a purely psychological or stress-related disorder. Clinical encounters with these patients typically require more time and effort, as well as clear, honest, and supportive communication (10,11).

A first-line intervention should involve discussion

of potential lifestyle modifications, including increased physical activity, dietary adjustments, and avoidance of symptom-triggering foods. Among dietary strategies, the low-FODMAP diet has gained popularity. This approach eliminates foods rich in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols, which exert osmotic effects, undergo fermentation, and promote gas production. Evidence for the efficacy of the FODMAP diet is based primarily on small-scale studies with limited generalizability and short-term outcomes. Nevertheless, many patients report symptomatic improvement with elimination of certain foods, which under the supervision of a clinical dietitian may represent an appropriate starting point for symptom management. Typically, such a diet involves a 3–6-week elimination phase, followed by assessment of response and gradual reintroduction of fermentable carbohydrates (9,10). Other less restrictive dietary approaches have also been studied, such as lactose elimination or the National Institute for Health and Care Excellence (NICE) diet, which recommends smaller, more frequent meals and avoidance of known triggers, including alcohol and caffeine. These strategies have demonstrated efficacy, although generally to a lesser degree than the low-FODMAP diet (3).

Interestingly, pharmaceutical formulations of peppermint oil have shown additional benefits, particularly in reducing bloating and abdominal pain. Enteric-coated capsules allow delivery of peppermint oil to the small and large intestine, and some studies have even demonstrated superior efficacy compared to antispasmodics or other pharmacologic agents for IBS (6,11). Similarly, combining turmeric essential oil with fennel has been reported to provide symptomatic improvement (12).

Soluble dietary fibres also have a beneficial role by improving stool regularity and consistency. However, intake should not exceed the recommended daily allowance for the general population (25–30 g/d). Soluble fibres, such as psyllium, are particularly useful because soluble fibres may improve symptoms of constipation and diarrhoea, while also reducing bloating and flatulence (6,10).

Microbiome, Probiotics, Prebiotics, and Postbiotics

One of the most extensively investigated topics in IBS research focuses on the intestinal microbiome and the potential role of probiotics. Studies involving the gut microbiota in IBS patients have demonstrated reduced microbial diversity, an increased abundance of specific bacterial strains with a concomitant decrease in non-pathogenic commensals, impaired resistance to pathogenic colonization, and altered mast cell activity that affects endocrine and neural signalling, thereby contributing to the pathogenesis underlying IBS (13). Ongoing studies aim to identify bacterial strains consistently associated with IBS but the results have been heterogeneous, reflecting the complexity of the gut ecosystem. To date, only one-third of the gut microbiota has been fully characterized (3). Jacobs et al. (14) recently reported increased abundance of *Bacteroides dorei*, *Actinomyces spp.*, *Streptococcus spp.*, *Eggerthella lenta*, and *Blautia hydrogenotrophica* among IBS patients in a racially and ethnically diverse cohort with reduced levels of *Faecalibacterium prausnitzii*, *Bacteroides thetaiotaomicron*, and *Bilophila wadsworthia* compared to healthy controls.

Other meta-analyses have reported decreased concentrations of *Bifidobacterium*, *Lactobacillus*, *F. prausnitzii*, and *Bacteroidetes* with an increased prevalence of *Firmicutes*, *Escherichia coli*, and *Enterobacter* (3). Altered bacterial metabolism has also been demonstrated, including an enhanced ability to utilize fermentable carbohydrates in IBS patients, which may help explain the clinical effectiveness of the low-FODMAP diet (14).

These variations have fuelled growing interest in the therapeutic use of probiotics. Current evidence suggests that probiotics may be most effective in IBS-D (6). Treatment beyond 12 w is not generally recommended if no symptomatic benefit is observed. The efficacy of probiotic preparations depends largely on strain composition, concentration, and, importantly, diversity; formulations with broader strain diversity are preferred (11).

Prebiotics are non-digestible carbohydrates that promote the growth or activity of beneficial gut bacteria. Examples of prebiotics include

fructooligosaccharides, galactooligosaccharides, and inulin. While IBS patients appear to have reduced intake of prebiotics, clinical data on the effectiveness of supplementation are limited and contradictory. Therefore, the routine use of prebiotics is not currently recommended in clinical guidelines (3,15).

Postbiotics, defined as bioactive compounds produced through microbial metabolism that exert beneficial effects on the host but do not meet the definition of probiotics or prebiotics, are another emerging therapeutic approach (16). Sodium butyrate is commonly used by patients with IBS in Central and Eastern Europe and has been studied as a postbiotic. Butyrate serves as an energy source for enterocytes, exerts anti-inflammatory effects, promotes cell proliferation, and inhibits apoptosis. A clinical study conducted in Slovenia reported significant improvement in the quality of life among IBS patients receiving sodium butyrate supplementation; no adverse effects occurred (17).

Personalized Pharmacologic Treatment

Pharmacologic therapy for patients with IBS is primarily symptom-oriented. Therefore, careful assessment of the predominant symptoms, classification into one of the subtypes (IBS-D, IBS-C, IBS-M, or IBS-U), and subsequent tailored treatment selection are essential (9,10).

Abdominal pain and bloating are often more bothersome in all IBS subtypes than altered bowel habits. These symptoms may be alleviated with antispasmodics (e.g., mebeverine), the gut-targeted antibiotic, rifaximin (rifaximin is only approved in Slovenia by the Hepatology Board [KOGE, University Medical Centre Ljubljana] and used exclusively for another indication), or in select cases, antidepressants. The choice of antidepressant has a significant role in treating IBS. Specifically, tricyclic antidepressants (TCAs) may induce constipation and are therefore preferable in patients with IBS-D, whereas selective serotonin reuptake inhibitors (SSRIs) may induce diarrhoea and are thus more appropriate in patients with IBS-C (9,10). Antidepressants should be considered as a second-line therapy, initiated at low doses (e.g., amitriptyline or doxepin [10 mg]), and gradually titrated to the maximum tolerated dose. Higher doses are associated with an increased risk of adverse effects (11).

Conventional analgesics, such as non-steroidal anti-inflammatory drugs (NSAIDs), paracetamol, or opioids, are generally ineffective in IBS-related pain and may even exacerbate symptoms (11). More promising results have been demonstrated with serotonin receptor modulators, such as alosetron, ramosetron, and ondansetron. Of serotonin receptor modulators, only ondansetron is currently available in Slovenia (4).

Loperamide remains a useful adjunct for diarrhoea-predominant IBS (9). Patients with constipation-predominant symptoms unresponsive to lifestyle modification and non-pharmacologic measures, may benefit from bulk-forming laxatives, such as psyllium, methylcellulose, corn fibre, or calcium polycarbophil, as well as osmotic agents containing polyethylene glycol. Lactulose-based laxatives should be used with caution because lactulose-based laxatives may aggravate bloating and abdominal pain. Other agents with proven efficacy include lubiprostone, linaclotide, tenapanor, and tegaserod, although these agents are not currently available in Slovenia (4,9).

Psychological Impact, Stress, and Psychotherapy

Approximately 44% of patients with IBS have been reported to have anxiety disorders and 25% have a diagnosis of depression. It remains unclear whether psychological processes exert a primary influence on gut function or whether disturbances in gastrointestinal function predispose to psychological co-morbidities; current evidence supports the latter (2). Referral for psychological or psychotherapeutic intervention should be considered in patients with a clear association between stress and IBS symptoms. Studies have demonstrated beneficial effects of various approaches (9). Cognitive–behavioural therapy has shown the greatest efficacy in patients with IBS, while hypnotherapy, dynamic psychotherapy, and relaxation-based interventions have also been associated with symptom improvement (4,10).

CONCLUSION

IBS is a disorder affecting an increasingly large proportion of the population with a corresponding rise in the number of healthcare professionals involved in IBS management. A stepwise approach to the diagnosis and treatment of IBS is recommended to ensure more effective and structured care. Although the clinical course in patients with IBS can often feel frustrating and at times without clear therapeutic solutions, research into the aetiology and treatment of IBS continues to advance. Patience and persistence among IBS patients and healthcare providers are required. Above all, it is essential to recognize that each patient seeks nothing more than meaningful relief of symptoms and an improved quality of life.

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Pristopi za ocenjevanje endokrine aktivnosti: od mehanističnih bioloških testov do poti neželenih izidov

Endocrine activity assessment approaches: From mechanistic bioassays to adverse outcome pathways

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Ključne besede:
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Izvleček

Namen: *Predstaviti celovit pregled sodobnih pristopov za odkrivanje in karakterizacijo endokrine aktivnosti kemikalij ter poudariti povezavo med mehanističnimi* in vitro *testi,* in vivo *modeli in regulatornimi okviri.*
Metode: *Primerjane so bile novejše raziskave na področju visokozmogljivega presejanja (HTS), validiranih OECD preskusnih smernic in efektivno zasnovanih bioloških testov. Pregled zajema reprezentativne teste za estrogene, androgene, ščitnične in steroidogene poti ter ocenjuje njihovo dopolnjevanje znotraj okvira Poti neželenih izidov (AOP). Posebna pozornost je bila namenjena integraciji podatkov, upoštevanju biološke uporabnosti in regulatornemu sprejemanju testov v okviru uredb REACH ter ameriškega programa* Endocrine Disruptor Screening Program (EDSP).
Rezultati: *Mehanistični testi, kot so*

Abstract

Purpose: *To provide an integrated overview of current approaches for detecting and characterizing endocrine activity in chemicals by emphasizing the relationship between mechanistic* in vitro *assays,* in vivo *models, and regulatory frameworks.*
Methods: *Recent developments in high-throughput screening (HTS) platforms, validated Organisation for Economic Co-operation and Development (OECD) test guidelines, and effect-based bioassays were critically compared. The review covers representative assays for estrogenic, androgenic, thyroid, and steroidogenic pathways and evaluates the complementarity within adverse outcome pathway (AOP) frameworks. Attention was given to data integration, bioavailability considerations, and regulatory adoption under the European Union Registration, Evaluation, Authorization,*

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YES, E-Screen, HeLa-9903 in H295R, omogočajo hitro, občutljivo in stroškovno učinkovito presejanje receptorsko posredovanih in steroidogenih učinkov, medtem ko in vivo sistemi, kot so indukcija vitelogenina, reprodukcijski testi pri vodnih bolhah, metamorfoza pri dvoživkah in testi na zebricah, zagotavljajo potrditev učinkov na ravni organizma. Integracija obeh pristopov v okviru AOP ter kvantitativne ekstrapolacije in vitro-in vivo (QIVIVE) *povečujejo napovedno zanesljivost testov. Kljub napredku v standardizaciji pa ostajajo izzivi, povezani z učinki metabolitov, med-laboratorijsko variabilnostjo in usklajenimi določitvami mejnih učinkov.*

Zaključek: *Znanost o preskušanju endokrine aktivnosti se usmerja v mehanistično bazirane pristope, ki zmanjšujejo porabo živali za testiranje in združujejo visokozmogljivo presejanje, računalniško modeliranje in efektivno zasnovano spremljanje. Usklajeni kriteriji za validacije in standardizirani izidi bodo dodatno okrepili regulatorno sprejemanje testov ter omogočili učinkovitejšo identifikacijo snovi z endokrino aktivnostjo v širšem kontekstu zdravja ljudi in okolja.*

and Restriction of Chemicals (REACH) and the US Environmental Protection Agency (EPA) Endocrine Disruptor Screening Program.

Results: *Mechanistic assays, such as YES, E-Screen, HeLa-9903, and H295R, provide rapid, sensitive, and cost-effective screening for receptor-mediated and steroidogenic effects. In contrast,* in vivo *systems, including vitellogenin induction,* Daphnia *reproduction, amphibian metamorphosis, and zebrafish assays, yield organism-level confirmation. Integrating both tiers within AOP and quantitative* in vitro-*to*-in vivo *extrapolation (QIVIVE) frameworks increases predictive reliability. Despite advances in standardization, challenges remain concerning metabolic competence, inter-laboratory variability, and harmonized effect thresholds.*

Conclusion: *Endocrine testing science is transitioning toward mechanistically anchored, animal-reduced strategies that combine HTS, computational modeling, and effect-based monitoring. Harmonized validation criteria and performance standards will further enhance regulatory acceptance and facilitate the efficient identification of substances with endocrine activity across both human health and environmental contexts.*

INTRODUCTION

Substances with endocrine effects, such as pesticides, plasticizers, pharmaceuticals, and personal care products, can be dispersed through wastewater effluents and environmental compartments, resulting in continuous, low-level exposures across ecosystems and the human population (1). Determining the effects of such compounds on the endocrine system is a complex process. The most comprehensive set of tests for identifying potential endocrine-disrupting chemicals (EDCs) is part of the United States Environmental Protection Agency (US EPA) screening program, with total testing costs exceeding one million USD per compound (2). *In vitro* and *in silico* high-throughput screening (HTS) procedures have become predominant in recent years due to the vast number of substances on the market because HTS procedures enable the collection of data for many compounds at a substantially lower cost (3). It is impossible to determine the influence of such a large array of compounds on all possible cellular receptors while considering the complexity and interconnections of biochemical processes in the body (4). The principle of these assays is to only identify the key molecular targets and reaction mechanisms through which endocrine disruptors can act, then to quantify the effects on these targets. The primary objective of all high-throughput approaches is to identify candidate substances for further testing in smaller, yet more reliable, assay systems, including *in vivo* studies (5). Therefore, the current article focuses on the detection and characterization of endocrine activity as part of the path to identifying EDCs.

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT FRAMEWORK

Each active substance is tested using a series of assays that address acute, sub-chronic, and chronic exposures in accordance with internationally accepted guidelines (6). Compounds are categorized into five levels based on the data obtained, as shown in Figure 1 (4). Existing data and non-testing information are evaluated in Level 1, including physicochemical properties, results from standard toxicity tests, *in silico* toxicity predictions, and pharmacokinetic modeling. Level 2 builds upon this foundation with *in vitro* assays that generate mechanistic data on endocrine activity in mammals and other species, such as binding affinity to estrogen (ER) or androgen (AR) receptors, activation of ER and AR, *in vitro* steroidogenesis, and activation of thyroid or retinoid receptors. These assays help identify molecular initiating and key events at the cellular level. Level 3 introduces first *in vivo* mechanistic assays which bridge molecular mechanisms with organismal responses. Representative tests include the uterotrophic and Hershberger assays, amphibian metamorphosis, short-term fish reproduction assays, and short-term endocrine activity tests in *Daphnia*. Together, Levels 2 and 3 form the mechanistic evidence tier, describing how a substance interacts with endocrine pathways and how these interactions manifest in short-term biological responses. Level 4 comprises more complex *in vivo* studies that provide apical data, capturing adverse effects mediated through endocrine-relevant targets. These include 28- and 90-d repeated-dose toxicity studies, one-generation reproductive toxicity studies, developmental and pubertal assays, and

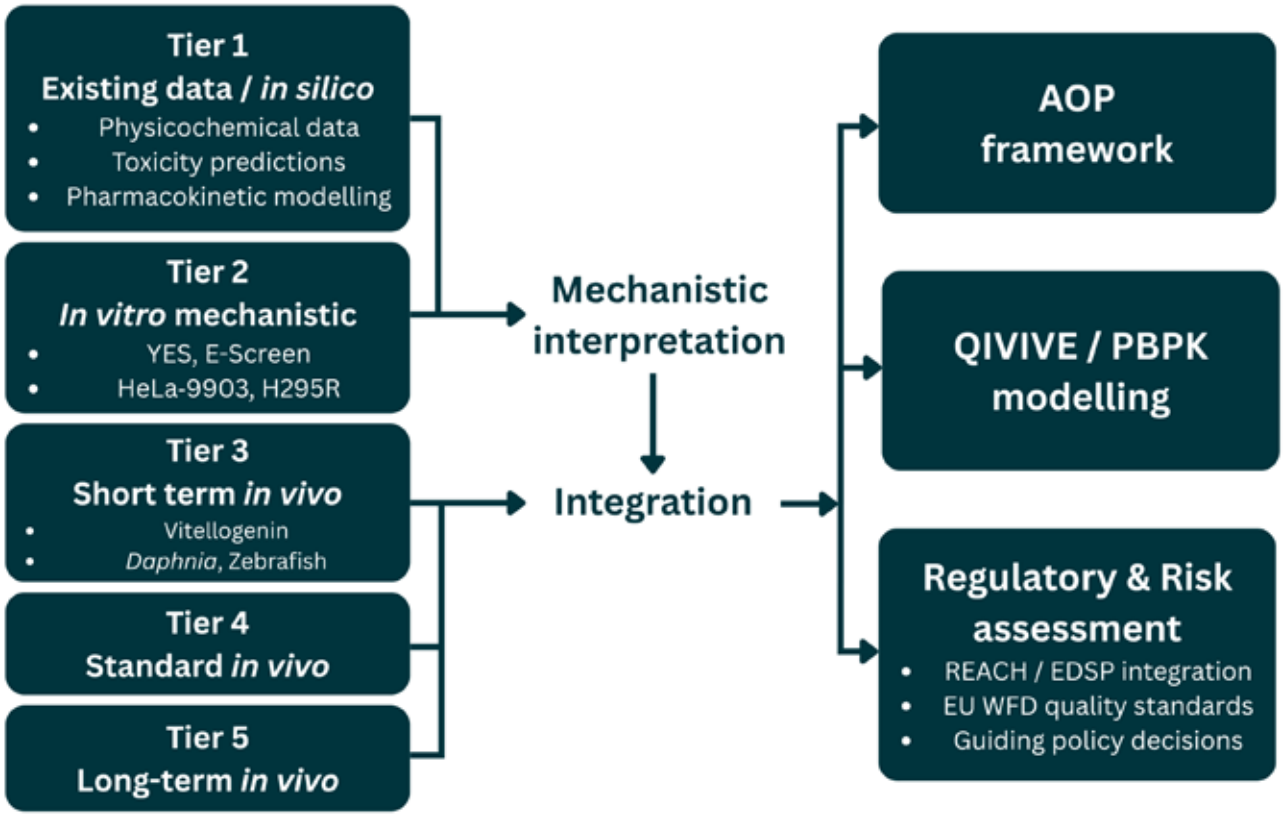

Figure 1. *Integrated endocrine testing and risk assessment framework. The tiered testing approach integrates into planning of adverse outcome pathway (AOP) frameworks, modelling of quantitative in vitro-to-in vivo extrapolation (QIVIVE), and physiologically based kinetic/pharmacokinetic (PBPK) models and ultimately regulatory requirements and risk assessments.*

chronic reproductive studies in fish and mammals. Level 5 represents the highest tier, encompassing long-term apical tests that integrate effects across multiple life-cycle stages and generations, such as the extended one- or two-generation reproductive toxicity studies and multi-generational fish or *Daphnia* tests.

Thus, the hierarchy connects mechanistic data (Levels 2–3), which explain how a substance interacts with the endocrine system, to apical data (Levels 4–5), which demonstrate the biological consequences these interactions cause at the organism and population levels. This finding is why comprehensive and robust Level 1 data are mandatory. Levels 2 and 3 are required if other studies raise suspicion. Level 4 and 5 studies provide increasing confidence in detecting endocrine effects. Level 5 is only required in specific ecotoxicological cases (6).

MECHANISTIC BASIS OF ENDOCRINE ACTIVITY

The primary site of action in the estrogenic hormonal system involves the interaction of endocrine-active substances with ERs, acting as either agonists or antagonists. Several molecular events are involved, as follows: ligand binding; receptor dimerization; nuclear translocation; formation of the transcriptional complex with cofactors; DNA binding; transcription; translation; post-translational modification; and cellular response. In addition to receptor-mediated signaling, several endocrine-disrupting compounds have been shown to affect DNA integrity and gene expression through oxidative stress, altered DNA methylation, and chromatin remodeling mechanisms (7). Because individual assays generally measure only one or a few of these processes, advanced multi-assay models, such as those in the US EPA Tox21 program, apply up to 18 complementary tests. Statistical integration of these results allows classification of a compound as an estrogenic agonist, antagonist, active through non-estrogenic pathways, or inactive (8). Optimization studies on approximately 1800 compounds have shown that comparable accuracy can be achieved with only 4 key assays, each targeting a distinct step in ER signaling (9). Developing models for evaluating effects on the androgenic system is even more complex due to the limited availability of reference compounds and technical challenges. Many chemicals act as AR antagonists and can induce cytotoxicity *in vitro*, producing false-positive results. Therefore, simultaneous cytotoxicity evaluation and co-exposure with known agonists are used to confirm antagonism and reduce false-positive results (10). Steroidogenesis is another critical endpoint. *In vitro* steroidogenesis assays and aromatase activity measurements reveal broader endocrine interference across axes, which helps to capture effects that single-receptor tests might miss (11). In addition to direct receptor binding, endocrine-active substances may interfere with intracellular signaling cascades, hormone transport, or metabolism. Crosstalk among nuclear receptor families, such as peroxisome proliferator-activated receptors (PPARs), aryl hydrocarbon receptor (AhR), and retinoid X receptor (RXR), can modulate endocrine responses without classic ER, AR, or thyroid hormone receptor (TR) activation. These non-receptor mechanisms expand the conceptual boundary of endocrine activity that can lead to endocrine disruption and should be incorporated into broader adverse outcome pathway (AOP) frameworks and HTS platforms to improve detection of indirect hormonal effects. This interconnection of different mechanisms is also the reason AOPs are a necessary framework for accurate effect determination.

Yeast Estrogen Screen

The yeast estrogen screen (YES) uses genetically modified *Saccharomyces cerevisiae* cells that express human ERα and contain an estrogen-responsive element linked to a reporter gene (typically *lacZ*). Upon activation, β-galactosidase is produced, converting a chromogenic substrate from red-to-yellow for spectrophotometric measurement. Modern versions of the YES use bioluminescent reporters (12). The assay detects agonism and competitive antagonism but is limited to ER-mediated activity, which is only a single component of the endocrine system. The YES is simple, low-cost, fast (< 24 h), sensitive (ng/L for estrogens), and robust to matrix effects. Multiple strains and commercial kits are available (13, 14).

E-screen

E-screen uses human MCF-7 cells to measure estrogen-induced proliferation. This cell line expresses ERα (and minor ERβ) and contains aromatase and 5α-reductase, enabling the conversion of androgens-to-estrogens. E-screen is generally more sensitive compared to yeast systems and detects agonists and antagonists. However, non-estrogenic mitogens can cause non-specific responses, which causes false-positive results. In addition, the assay can last up to 7 d even though automation and parallel analysis of many samples are feasible. Accelerated readouts include estrogen-responsive genes, such as *pS2* mRNA, which is detectable within hours (15, 16). Compounds may test negative in this assay yet show activity in more complex *in vivo* assays [e.g., vitellogenin (VTG)] (17).

hERα-HeLa-9903

The hERα-HeLa-9903 transcriptional activation assay, which is part of US EPA screening, measures ERα-driven luciferase expression in engineered HeLa cells (18). Bioluminescence correlates with receptor activation with estradiol serving as the positive control. The assay identifies agonists and provides mechanistic insight but cannot predict whole-organism outcomes influenced by metabolism, endocrine feedback, receptor crosstalk, and pharmacokinetic processes. In addition, some compounds may cause false-positive results at high concentrations by directly activating luciferase without receptor binding (19). Standardized commercial kits improve inter-laboratory comparability (20, 21).

VTG

VTG is an egg-yolk precursor synthesized in the liver of adult females in most oviparous species. VTG is induced by estradiol. Induction of VTG in males serves as a sensitive biomarker of estrogen exposure (22). VTG is commonly quantified in fish, such as *Pimephales promelas*, as mRNA (indicating a rapid response) or protein (indicating long-term exposure) using an enzyme-linked immunosorbent assay (ELISA) or liquid chromatography-mass spectrometry (LC-MS). ELISA is a practical and sensitive method, although results can vary depending on the antibodies and standards used. Standardized kits recommended by the guideline of the Organisation for Economic Co-operation and Development (OECD) number 229 help mitigate this variability, although only partially (23, 24). Non-lethal sampling from epidermal mucus is also possible (25).

Daphnia

Daphnia magna, a small planktonic crustacean, reproduces by cyclical parthenogenesis, which allows for rapid multigenerational observation. Chronic tests monitor survival, size, morphology, fecundity, and sex ratio under controlled conditions. The OECD test number 211 (21-d reproduction test with at least 10 individuals) is used and considered the gold standard for assessing chronic reproductive toxicity. However, *Daphnia* lacks classical vertebrate steroid receptors, meaning the endocrine signaling pathways differ substantially from fish or mammals and are therefore not suitable for identifying specific endocrine molecular mechanisms (26, 27).

Zebrafish

Zebrafish (*Danio rerio)* offer a human-relevant *in vivo* system due to transparent embryos, rapid organogenesis, high fecundity, and approximately 70% genome homology with humans, which is why zebrafish partly bridge the gap between mechanistic *in vitro* assays and vertebrate testing (28). Experimental endpoints include reproductive, developmental, histologic, and behavioral measures, such as swimming activity (29). A variant of the zebrafish system, the EASZY assay, utilizes cyp19a1b-GFP transgenic embryos and is currently under evaluation by the OECD as a method for detecting endocrine-active substances. The main limitations of the zebrafish system lie in the fact that the interpretation of results is influenced by developmental stage, duration of exposure, and non-specific toxicity, while the tests remain comparatively resource-intensive and require careful standardization (30, 31).

Thyroid and Steroidogenic Pathways

The thyroid axis regulates metabolism, growth, and neurodevelopment through thyroxine (T4) and

triiodothyronine (T3). Endocrine-active chemicals disrupt this axis by inhibiting thyroid peroxidase (TPO), altering sodium-iodide symporter (NIS) function and iodide uptake, and competing for transport proteins, such as transthyretin, modulating deiodinases (D1-D3), or interacting with nuclear thyroid hormone receptors (TRα/β). Regulatory test systems capture these modes of action at multiple biological levels. The amphibian metamorphosis assay [OECD TG 231] (32) quantifies thyroid-dependent developmental timing in *Xenopus laevis* tadpoles using hind limb growth and stage progression as sensitive endpoints. Fish-based assays, such as the fish short-term reproduction assay [OECD TG 229] can include thyroid-relevant biomarkers with reproductive measures (33). Similarly, steroidogenesis is closely linked to thyroid status and is a major route of interactions with endocrine activity. The H295R steroidogenesis assay [OECD TG 456] (34) uses human adrenocortical carcinoma cells to quantify altered estradiol and testosterone production after chemical exposure, while targeted enzyme assays (aromatase/CYP19A1 and 17β-HSD) identify specific nodes affected (35). Together, these assays enable mechanistic weight-of-evidence determinations that support translation to organism-level outcomes when integrated with toxicokinetic data. However, interspecies variability in thyroid regulation and limited metabolic capacity in steroidogenic models underscore the need for complementary endpoints and integrative interpretation within the AOP and extrapolation frameworks to ensure reliable translation from mechanistic data to organism-level effects.

MIXTURES, ENVIRONMENTAL RELEVANCE, AND BIOAVAILABILITY

Environmental exposures rarely involve single chemicals. Instead, organisms encounter mixtures with diverse modes of action at the sub-ng/L-to-µg/L levels (36). Mixture effects can be additive (concentration addition), independent (response addition), or antagonistic/synergistic and are strongly influenced by bioavailability and metabolism. Classical *in vitro* tests typically use nominal concentrations and lack metabolic competence, which may misrepresent internal dosimetry and active metabolites (37). Bridging assays with exposure science improves realism. Specifically, passive samplers (e.g., silicone sheets and disks) and polar organic chemical integrative sampler (POCIS) devices provide time-integrated extracts that can be assayed directly (effect-based monitoring). Effect results can be expressed as equivalency metrics (e.g., estradiol equivalents [E2eq] for ER activity or dihydrotestosterone equivalents [DHTeq] for AR), allowing comparison to effect-based trigger values proposed for water quality assessment (38). Coupling bioassays with toxicokinetic modeling and quantitative structure-property relationship-based bioavailability estimates refines mixture interpretation, while fractionation (HPLC or SPE) can deconvolute mixture drivers. Ultimately, mixture-aware assessment benefits from combining effect-directed analysis with targeted high-resolution mass spectrometry (HRMS) to link bioactivity to toxicologically active substances (39).

DATA INTEGRATION AND ADVERSE OUTCOME PATHWAYS

Large-scale programs, such as Tox21 and ToxCast, generate HTS data across hundreds of receptors and enzymes relevant to endocrine biology. To translate these data into decisions, the AOP framework links molecular initiating events to key events and adverse outcomes at the organism or population level (40). Quantitative AOPs (qAOPs) further incorporate dose-response and time-to-event information, enabling predictions under realistic exposure scenarios. Integration pipelines combine HTS potency metrics (e.g., AC_{50} and area under the curve), cytotoxicity filtering, and chemotype alerts with quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE) and physiologically based kinetic/pharmacokinetic (PBK/PBPK) models to estimate external doses that produce equivalent internal target effects (41). Machine learning models trained on curated HTS and apical datasets support read-across and prioritization, while flagging uncertainty (42). However, these computational methods do not replace validated test

Table 1. *Overview of Representative Assays for Assessing Endocrine Activity*

Assay / System	Biological Level	Primary Endpoint(s)	Typical Duration	OECD Test Guideline	Approximate Cost / Throughput	Notes / Strengths
YES (Yeast Estrogen Screen)	*In vitro* (yeast, estrogen receptor - ERα)	Estrogen receptor activation (agonism/ antagonism)	< 1 day	-	Low / High	Simple, rapid, inexpensive; limited to ER-mediated effects
E-Screen (MCF-7)	*In vitro* (human cells)	Estrogen-dependent proliferation	3-7 days	-	Moderate / Moderate	Sensitive; detects genomic & non-genomic activity; some nonspecificity
hERα-HeLa-9903	*In vitro* (human cells)	ERα transcriptional activation (luciferase reporter)	≤ 1 day	TG 455	Moderate / High	Standardized; detects agonists/antagonists; no metabolism
H295R Steroidogenesis	*In vitro* (adrenocortical cells)	Estradiol/testosterone synthesis alteration	1-2 days	TG 456	Moderate / Moderate	Mechanistic assay targeting steroid biosynthesis
Vitellogenin (VTG)	*In vivo* (fish)	Plasma/hepatic VTG induction	7-21 days	TG 229 / 230	High / Low	Sensitive; biologically relevant; species dependent
Daphnia Reproduction Test	*In vivo* (crustacean)	Survival, fecundity, sex ratio	21 days	TG 211	Moderate / Low	Chronic ecotoxicological relevance
Amphibian Metamorphosis Assay	*In vivo* (amphibian)	Developmental stage, hind-limb growth	21 days	TG 231	High / Low	Specific for thyroid activity; regulatory acceptance
Zebrafish (EASZY, etc.)	*In vivo (*fish)	Transgenic ER-responsive gene expression, reproduction	7-30 days	TG 234 / in validation	High / Moderate	High-content developmental endpoints; suited for screening

guidelines but enhance prioritization and reduce animal use by focusing *in vivo* testing on the most plausible risks.

COMPARATIVE OVERVIEW OF METHODS

The scope of endocrine testing ranges from mechanistic precision to ecologic relevance, as presented in Table 1. *In vitro* systems, such as YES, HeLa-9903, and H295R, provide rapid, mechanistically specific information, while *in vivo* assays (VTG induction, *Daphnia* 21-d reproduction, zebrafish embryo tests, and amphibian metamorphosis) capture whole-organism adversity. Integrating both levels in a stepwise or evidence-based sequence enables efficient screening, while maintaining biological relevance. Validation and regulatory acceptance vary, as follows ER/AR transcriptional activation assays align with OECD TG 455 principles; VTG is a well-established biomarker in fish population models; and OECD TG 211 (*Daphnia*) and TG 231 (amphibian metamorphosis) are widely recognized for chronic and thyroid-specific endpoints. Cost-time trade-offs favor a tiered strategy, which first deploys HTS to map mechanistic space and derive potency rankings, followed by targeted *in vivo* tests to confirm adversity at relevant life stages. Effect-based monitoring, when feasible, is used to connect laboratory potency with field exposures. Tabulating sensitivity, specificity, typical runtime, and indicative costs per assay family can further support study design and regulatory submissions.

FUTURE PERSPECTIVES

Endocrine toxicology is shifting toward animal-reduced, mechanism-driven assessment. Emerging tools include 3D organoids and microphysiologic systems (organ-on-chip) that reproduce human tissue architecture and hormone feedback loops, single-cell and spatial omics that reveal hormone-responsive cell states, and label-free biosensors that enable continuous ER/AR/TR signaling readouts (43). HRMS (untargeted and suspect screening) accelerates effect-directed analysis, while computational docking and deep learning improve virtual screening for receptor binding and enzyme inhibition (44). Standardization efforts by the OECD and the International Organization for Standardization (ISO) with open data infrastructures, such as the CompTox Chemicals Dashboard, are crucial for interoperability. Future priorities include establishing performance standards for novel models, routinely embedding toxicokinetics/QIVIVE, and harmonizing effect-based trigger values across jurisdictions to enable bioassays to inform regulatory thresholds with conventional chemical monitoring (45).

REGULATORY AND RISK ASSESSMENT IMPLICATIONS

Regulatory decision-making increasingly integrates mechanistic and apical evidence within transparent frameworks. REACH and the Biocidal Products Regulation in the European Union use criteria for identifying endocrine disruptors, which are supported by guidance from the European Chemicals Agency (ECHA)/European Food Safety Authority (EFSA). Annex XV dossiers and weight-of-evidence approaches encourage combining *in vitro* mechanistic data (e.g., ER/AR transactivation assays and H295R) with *in vivo* adversity data (e.g., TG 231 and TG 211) and toxicokinetic information (46). The Endocrine Disruptor Screening Program in the US leverages ToxCast/Tox21 data for Tier 1 screening and uses Tier 2 tests for confirmation (47). Water-quality management bodies increasingly use effect-based monitoring with bioassays (ER-CALUX/YES, AR-CALUX, and H295R) with targeted chemical analysis, guided by emerging effect-based trigger values in technical reports under the EU Water Framework Directive (48). The adoption of the AOP framework supports structured evidence evaluation across jurisdictions, while QIVIVE/PBPK models help translate *in vitro* potency into external exposure benchmarks, such as derived no-effect levels or environmental quality standards. Citing specific OECD Test Guidelines (e.g., TG 229, 231, 234, and 456) and providing links to official documents for submissions strengthens traceability and facilitates regulatory review. Despite major progress, several limitations persist. Many *in vitro* systems lack metabolic activation, potentially underestimating the activity of biotransformation-dependent disruptors. Inter-laboratory variability, differences in reference materials, and incomplete data integration frameworks still constrain cross-study comparability. Furthermore, effect-based trigger values and potency thresholds for bioassays remain inconsistently defined across regions (49). Future harmonization of assay validation, the inclusion of metabolic effects, and the establishment of standardized effect thresholds are essential to achieve regulatory convergence and enhance predictive power.

CONCLUSIONS

The described assays together form a comprehensive toolkit for identifying and characterizing endocrine-active substances. A robust assessment of endocrine activity requires the detection of receptor-level interactions, as well as the integration of toxicokinetic, organismal biology, and ecologic relevance. Combining *in vitro* mechanistic assays, *in vivo* functional studies, exposure science, and computational modeling within the AOP framework provides an efficient, predictive, and ethically sustainable path forward.

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Zgodnji pooperativni izidi v retrospektivni primerjavi med metodo po Lichtensteinu in Rutkow-Robbins metodi pri operaciji dimeljske kile

Early postoperative outcomes in a retrospective comparison between Lichtenstein repair method and Rutkow-Robbins repair method for inguinal hernia repair

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Ključne besede:
ingvinalna hernia, Lichtenstein, Rutkow-Robbins, polipropilenska mrežica, pooperativni izidi

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Izvleček

Namen: *Namen te retrospektivne študije je bil raziskati razlike v pooperativnih izidih pri bolnikih z dimeljsko kilo, zdravljenih z Lichtensteinovo ali Rutkow-Robbinsovo hernioplastiko.*
Metode: *Retrospektivno je bilo analiziranih 90 moških bolnikov, operiranih po bodisi Lichtensteinovi bodisi Rutkow-Robbinsovi metodi. Opazovane spremenljivke so bile pooperativni zapleti, in sicer zastajanje urina, zgodnja in pozna pooperativna krvavitev, bolečina in uporaba analgetikov, okužba rane in mrežice, skrotalni hematom in atrofija testisa, čas do mobilizacije bolnika ter ponovitev kile.*
Rezultati: *Med skupinama, operiranima po ali Lichtensteinovi ali Rutkow-Robbinsovi metodi, statistično značilnih razlik v pojavnosti spremljanih pooperativnih zapletov ni bilo ugotovljenih ($p > 0{,}005$).*
Zaključek: *Ta študija je pokazala, da med skupinama, operiranima po*

Abstract

Purpose: *The purpose of this retrospective study was to determine the difference in postoperative outcomes in inguinal hernia patients treated with a Lichtenstein or Rutkow-Robbins hernioplasty.*
Methods: *Ninety male patients who underwent a Lichtenstein or Rutkow-Robbins hernioplasty were retrospectively analysed. The observed variables were postoperative outcomes, specifically urinary retention, early and late postoperative bleeding, pain and analgesic usage, wound and implant infection, scrotal hematoma and testicular atrophy, patient mobilization time, and hernia recurrence.*
Results: *No statistically significant differences in the incidence of postoperative outcomes were detected between the Lichtenstein and Rutkow-Robbins hernioplasty groups ($P > 0.005$).*

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Lichtensteinovi metodi in Rutkow-Robbinsovi metodi, v pooperativnih izidih statistično značilnih razlik ni.

Conclusion: *No statistically significant differences in postoperative outcomes were detected between patients undergoing Lichtenstein and the Rutkow-Robbins hernioplasty repairs.*

INTRODUCTION

Inguinal hernia repair is among the most frequently performed surgeries globally with over 20 million operations performed each year (1). Despite advances in surgical techniques and medical technologies, no unified agreement exists regarding the optimal approach for repairing inguinal hernias (1). In 1986 Lichtenstein introduced a tension-free mesh repair technique utilising a polypropylene mesh to reinforce the fascia transversalis. This innovation was followed by the development of the mesh plug repair technique, which has since gained popularity and shown favourable outcomes in certain US centres (2). The Lichtenstein repair method (LRM) is considered relatively straightforward compared to the Rutkow-Robbins repair method (RRRM) because the LRM involves placement of a polypropylene mesh at the site of the hernia defect (3,4). The primary objective of successful hernia repair is to minimise complications and recurrence, while ensuring a quick recovery with minimal discomfort that enables patients to resume normal activities as soon as possible. Inguinal hernias represent 75% of abdominal wall hernias with a 27% and 3% lifetime risk of recurrence among males and females, respectively. Some patients have characteristics that increase the risk of postoperative complications and hernia recurrence (5).

Few studies have compared the efficacy of different types of hernia repairs. Karaca et al. (6) compared the Lichtenstein and Gilbert double-layer techniques in treating patients with inguinal hernia and found that the LRM was superior with respect to costs and venous blood flow with no other significant differences.

In this study, we compared The postoperative complications and recurrence between two hernia repair methods (LRM with a mesh and RRRM with a mesh and plug) in patients with unilateral inguinal hernias were compared in the current study.

MATERIALS AND METHODS

The current study was designed as a retrospective analysis of postoperative complications in 90 male patients diagnosed with inguinal hernias between 2016 and 2018, none of whom had undergone prior surgical intervention. Patients < 18 years of age, as well as patients with bilateral inguinal, recurrent, or incarcerated hernias, were excluded. The patients underwent an LRM or RRRM repair (n = 45 each). The same surgeon performed all surgeries and the mean follow-up period was two years. The two groups of patients were evaluated for age, body mass index (BMI), co-morbidities, American Society of Anaesthesiology (ASA) score, and hernia type, as classified according to the Nyhus system based on superficial ultrasonography and hernia location. Patients were admitted to the hospital 1 day prior to surgery and preoperative medications were administered 30 min before surgery. Inguinal hair was shaved in the operating room using electric clippers immediately before surgery. General and spinal anaesthesia was used in 22 and 68 patients, respectively. All patients received prophylactic antibiotics (cefazoline) and antithrombotic therapy (nadroparin). The criteria for hospital discharge included subjective wellbeing, no major postoperative complications (hematoma, urinary retention, seroma, and wound infection), limited mobility (i.e., ability to ambulate to and from the bathroom independently), and pain controlled with oral analgesics. Postoperative outcomes, including complications (pain, as measured

on a visual analogue scale (VAS) in which 0 = no pain and 10 = unbearable pain), analgesic requirements, urinary retention, early and late bleeding, scrotal hematoma, testicular atrophy, wound or implant infection, early and late patient mobilisation, and hernia recurrence, were compared. Patients were followed for 2 years postoperatively per the clinical practice in our Department and because nearly 25% of hernia recurrences occur during this time (7).

Surgical technique

The operative field was shaved in the operating room immediately prior to surgery following the induction of general anaesthesia or administration of spinal anaesthesia. Antibiotic prophylaxis was given (cefazoline). Skin disinfection was performed using 10% povidone-iodine and the surgical area was covered with sterile drapes.

The operation in the LRM group began with an oblique incision in the inguinal region, approximately 5 cm long, starting approximately 4 cm medial to the inguinale ligament. Fatty and aponeurotic tissues overlying the aponeurosis of the obliquus externus muscle were dissected sharply to expose the underlying aponeurosis. The aponeurosis was

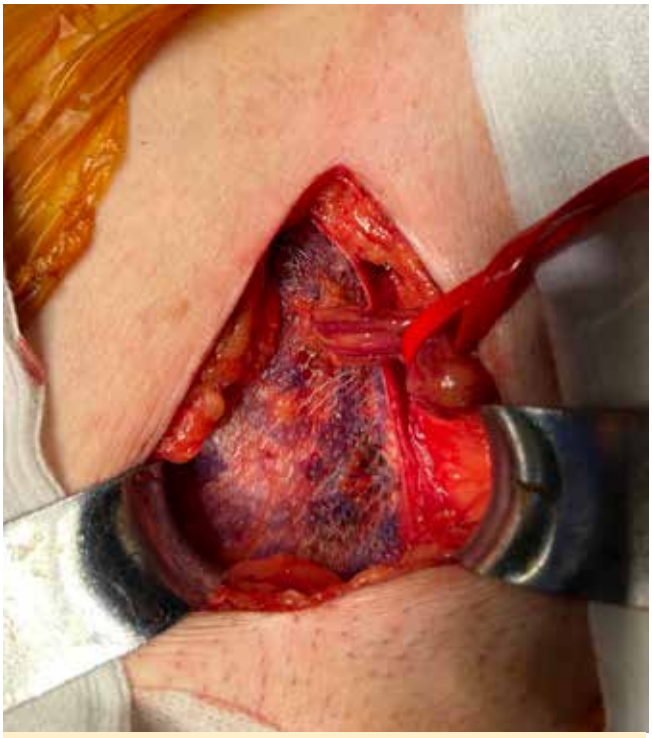

Figure 1. *An inguinal hernia repaired using the Lichtenstein repair method with polypropylene mesh.*

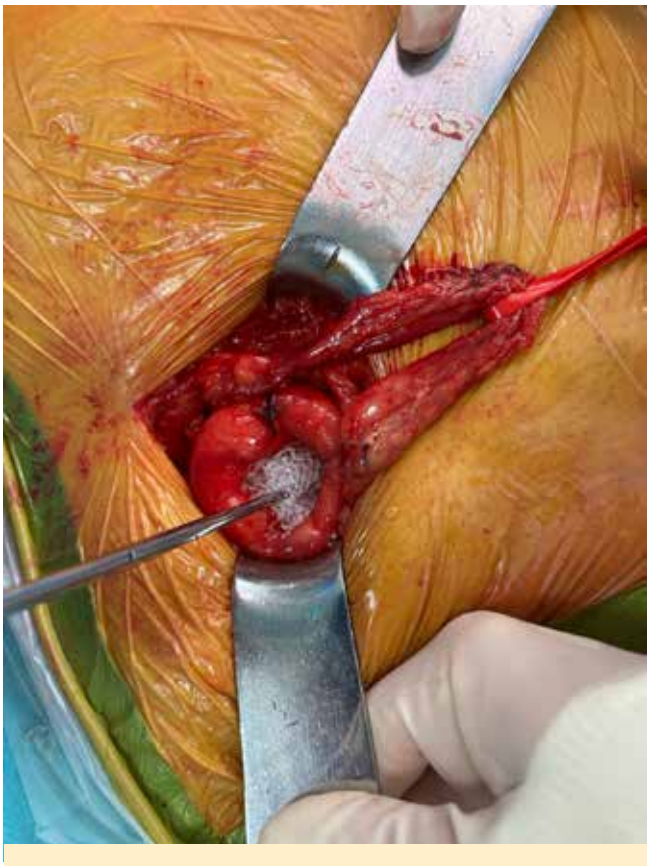

Figure 2. *An inguinal hernia repaired using the Rutkow-Robbins repair method with mesh plug.*

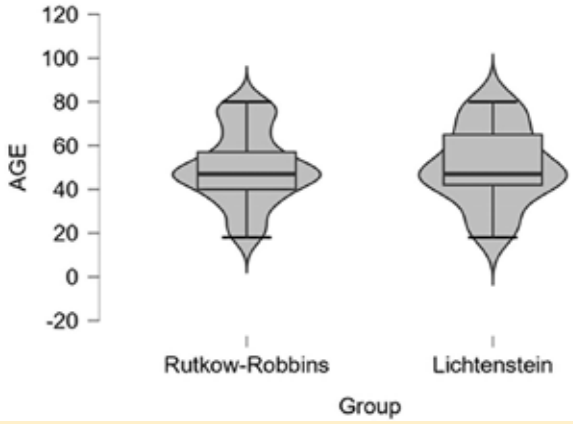

Figure 3. *Box-plot graph of patient age for both groups.*

then incised towards the pubic tubercle.
Then, cremaster fibres were detached from the aponeurosis above the pubic tubercle. Care was taken to preserve the spermatic cord and the genital branch of the genitofemoral nerve. The spermatic cord was placed on a thin rubber drain and the region from the pubis-to-the internal inguinal ring was examined. The ilioinguinal nerve was also preserved during the dissection. The hernial sac was identified and a herniotomy was performed. The hernia sac was then ligated at the level of the neck of the hernia, then excised. A pre-prepared polypropylene mesh was placed and adjusted over the fascia transversalis and fixed in place using tissue glue (Figure 1).
The dissection to the hernia was performed in the RRRM groups using the same technique as the LRM group. The hernial sac was not excised, rather inverted into the abdominal cavity and a mesh plug was then inserted into the internal inguinal ring, leaving the narrow end within the ring. The flat mesh was positioned over the transverse fascia covering the plug and secured with tissue glue. This method was

Table 1: *Patient age, operative time, and BMI for both groups and normality and equality of variances tests for age, operative time, and BMI for both groups*

	AGE		OP. TIME		BMI	
	RRRM	LRM	RRRM	LRM	RRRM	LRM
Valid	45	45	45	45	45	45
Missing	0	0	0	0	0	0
Median	47.000	47.000	55.000	55.000	21.000	23.000
Mean	49.644	50.267	57.311	53.689	21.200	23.244
Std. Deviation	16.561	17.155	8.179	7.245	2.668	2.932
IQR	17.000	23.000	15.000	6.000	2.000	3.000
Minimum	18.000	18.000	45.000	40.000	17.000	18.000
Maximum	80.000	80.000	75.000	73.000	26.000	30.000

Test of Normality (Shapiro-Wilk)		
Residuals	W	p
AGE	0.957	0.004
OP. TIME	0.972	0.050
BMI	0.964	0.014

Test of Equality of Variances (Brown-Forsythe)				
	F	df1	df2	p
AGE	0.146	1	88	0.703
OP. TIME	1.066	1	88	0.305
BMI	0.259	1	88	0.612

Significant results suggest a deviation from normality

Table 2: *Both study groups divided by type of anaesthesia used for surgery*

Group	Type of anesthesia	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	General	12	26.667	26.667	26.667
	Spinal	33	73.333	73.333	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	General	10	22.222	22.222	22.222
	Spinal	35	77.778	77.778	100.000
	Missing	0	0.000		
	Total	45	100.000		

Table 3: *Frequency table for urinary retention in both groups with contingency table and chi-squared result for urinary retention*

Frequencies for urin. reten.

Group	Urin. reten.	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	39	86.667	86.667	86.667
	Yes	6	13.333	13.333	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	41	91.111	91.111	91.111
	Yes	4	8.889	8.889	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
urin. reten.	**RRRM**	**LRM**	**Total**
No	39	41	80
Yes	6	4	10
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	0.450	1	0.502
N	90		

Note: Each cell displays the observed counts.

used for all patients in the RRRM group (Figure 2) (3). After the hernia repair the layers were closed anatomically in both groups. The aponeurosis of the external oblique muscle was sutured with 2-0 vicryl sutures. Subcutaneous tissue and skin were closed using 2-0 vicryl and 3-0 nylon sutures, respectively. The surgical site was cleansed with saline solution and a bandage was applied to complete the procedure.

Statistical analysis

The data collected in this study were analysed using the SPSS software package (version 20.0; SPSS

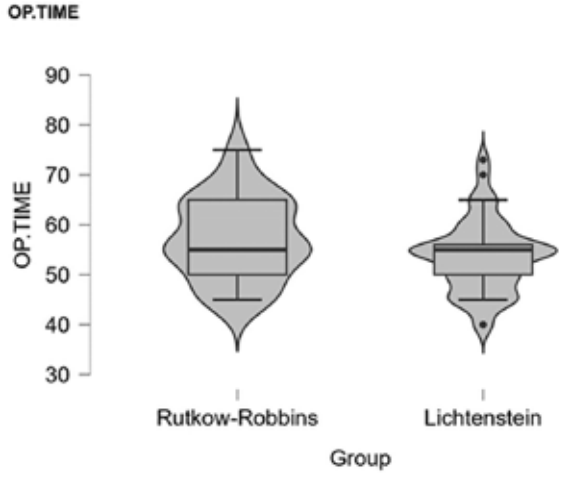

Figure 4. *Box-plot graph of operative time for both groups.*

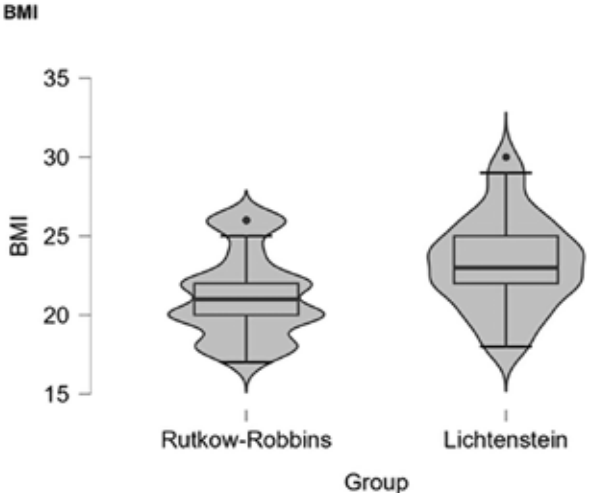

Figure 5. *Box-plot graph of operative time for both groups.*

Table 4: *Frequency table for early and late bleeding in both groups with contingency table and chi-squared result for both groups*

Frequencies for early bleed

Group	early bleed	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	40	88.889	88.889	88.889
	Yes	5	11.111	11.111	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	42	93.333	93.333	93.333
	Yes	3	6.667	6.667	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Early bleed	**RRRM**	**LRM**	**Total**
No	40	42	82
Yes	45	3	8
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X2	0.549	1	0.459
N	90		

Frequencies for late bleed

Group	Late bleed	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	43	95.556	95.556	95.556
	Yes	2	4.444	4.444	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	44	97.778	97.778	97.778
	Yes	1	2.222	2.222	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
late bleed	**RRRM**	**LRM**	**Total**
No	43	44	87
Yes	2	1	3
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	0.345	1	0.557
N	90		

Statistics for Windows). Descriptive statistics, including frequency and percentage distributions, are presented. The Mann-Whitney U test was used for variables that did not follow a normal distribution, as determined by a normality test, to compare the two groups. The Wilcoxon signed-rank test was utilised for pre- and post-measurement comparisons of non-normally distributed variables. A significance threshold of 0.005 was applied for all analyses. Differences were considered statistically significant at a $P < 0.005$, while a $P > 0.005$ indicated no significant difference.

A chi-square test was used to assess dependencies between variables with the same significance level of 0.005. A P-value below this threshold indicated

Table 5: *Frequency table for wound infection and scrotal hematoma in both groups with contingency table and chi-squared result for both groups*

Frequencies for wound inf-seroma

Group	Wound inf-seroma	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	41	91.111	91.111	91.111
	Yes	4	8.889	8.889	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	42	93.333	93.333	93.333
	Yes	3	6.667	6.667	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Wound inf-seroma	**RRRM**	**LRM**	**Total**
No	41	42	83
Yes	4	3	7
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	0.155	1	0.694
N	90		

Frequencies for scrotal hemat.

Group	Scrotal hemat.	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	39	86.667	86.667	86.667
	Yes	6	13.333	13.333	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	40	88.889	88.889	88.889
	Yes	5	11.111	11.111	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Scrotal hemat.	**RRRM**	**LRM**	**Total**
No	39	40	79
Yes	6	5	11
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	0.104	1	0.748
N	90		

significant dependency, whereas a value above this threshold indicated no significant dependency.

All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research.

RESULTS

Among 90 patients with inguinal hernias, 45 each underwent a Lichtenstein or Rutkow-Robbins hernioplasty and all received a polypropylene graft. The same surgeon operated on all patients in the same

Table 6: *Frequency table for testicular atrophy in both groups and contingency table for both groups*

Frequencies for testicular atrophy

Group	Testicular atrophy	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	No	44	97.778	97.778	97.778
	Yes	1	2.222	2.222	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	No	45	100.000	100.000	100.000
	Yes	0	0.000	0.000	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Testicular atrophy	**RRRM**	**LRM**	**Total**
No	44	45	89
Yes	1	0	1
Total	45	45	90

hospital. The characteristics of the LRM group were as follows: mean age, 50.3 years; male gender; mean BMI, 23.2 kg/m2; ASA score, 1 or 2; most patients had Nyhus type two inguinal hernias; and mean operative time, 53.7 min. The characteristics of the RRRM group were as follows: mean age, 49.6 years; male gender; mean BMI, 21.2 kg/m2; ASA score, 1 or 2; most patients had Nyhus type two inguinal hernias; and mean operative time, 57.3 min. Normality and equality of variances tests were performed to assess both groups (Table 1) and box-plot graphs were also created (Figures 3-5). Twelve patients in the RRRM group received general anaesthesia and 33 received spinal anaesthesia, while 10 received general anaesthesia and 35 received spinal anaesthesia in the LRM group (Table 2). Four patients (8.9%) in the LRM group and six patients (13.3%) in the RRRM group developed urinary retention (Table 3). Bleeding in the first 24 h after the procedure occurred in three (6.7%) and five patients (11.1%) in the LRM and RRRM groups, respectively. Bleeding > 24 h after the procedure occurred in one (2.2%) and two patients (4.4%) in the LRM and RRRM groups, respectively (Table 4). Wound and implant infections or seromas occurred in three patients (6.7%) in the LRM group and four patients (8.9%) in the RRRM group. Five patients (11.1%) in the LRM group and six patients (13.3%) in the RRRM group developed scrotal hematomas (Table 5). Testicular atrophy occurred in one patient (2.2%) in the RRRM group but in none of the patients in the LRM group (Table 6). No patients in either group developed a recurrent hernia. Twenty-two (48.9%), 14 (31.1%), and nine patients (20%) in the LRM group rated the pain intensity as0–3, 4–7, and 8–10, respectively. Fourteen (31.1%), 18 (40%), and 13 patients (28.9%) in the RRRM group rated the pain intensity as 0–3, 4–7, and 8–10, respectively. An analgesic dose of 5 g of metamizole per day was not sufficient for any patient. Fifteen patients (33.3%) in the LRM group required < 7.5 g of metamizole compared to nine patients (20%) in the RRRM group. Twenty-one patients (46.7%) in the LRM and RRRM groups required > 7.5 g of metamizole. Piritramide was used in nine patients (20%) in the LRM group and 15 patients (33.3%) in the RRRM group (Table 7). Nineteen patients (42.2%) in the LRM group were ambulating within the first 24 h after surgery compared to 15 patients (33.3%) in the RRRM group. Twenty-six (57.8%) and 30 patients (66.7%) were ambulating > 24 h after the procedure in the LRM and RRRM groups, respectively (Table 8). Thirty-four (75.6%), 9 (20%),and two patients (4.4%) in the LRM

Table 7: *Frequency table for pain assessment on VAS and dose of required analgesics in both groups with contingency table and chi-squared result for both groups*

Frequencies for pain assesment VAS

Group	Pain assesment VAS	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	1-3	14	31.111	31.111	31.111
	4-7	18	40.000	40.000	71.111
	8-10	13	28.889	28.889	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	1-3	22	48.889	48.889	48.889
	4-7	14	31.111	31.111	80.000
	8-10	9	20.000	20.000	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Pain assesment VAS	**RRRM**	**LRM**	**Total**
1-3	14	22	36
4-7	18	14	32
8-10	13	9	22
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X2	3.005	2	0.223
N	90		

Frequencies for dose of analgetics

Group	Dose of analgetics	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	Metamizole <7.5g	9	20.000	20.000	20.000
	Metamizole >7.5g	21	46.667	46.667	66.667
	Piritramide 22.5mg	15	33.333	33.333	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	Metamizole <7.5g	15	33.333	33.333	33.333
	Metamizole >7.5g	21	46.667	46.667	80.000
	Piritramide 22.5mg	9	20.000	20.000	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Dose of analgetics	**RRRM**	**LRM**	**Total**
Metamizole <7.5g	9	15	24
Metamizole >7.5g	21	21	42
Piritramide 22.5mg	15	9	24
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X2	3.000	2	0.223
N	90		

Table 8: *Frequency table for early and late mobilization in both groups with contingency table and chi-squared result for both groups*

Frequencies for early mobilization

Group	Early mobilization	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	1-3	14	31.111	31.111	31.111
	4-7	18	40.000	40.000	71.111
	8-10	13	28.889	28.889	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	1-3	22	48.889	48.889	48.889
	4-7	14	31.111	31.111	80.000
	8-10	9	20.000	20.000	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Early mobilization	**RRRM**	**LRM**	**Total**
1-3	14	22	36
4-7	18	14	32
8-10	13	9	22
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	3.005	2.	0.223
N	90		

Frequencies for late mobilization

Group	Dose of analgetics	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	Metamizole <7.5g	9	20.000	20.000	20.000
	Metamizole >7.5g	21	46.667	46.667	66.667
	Piritramide 22.5mg	15	33.333	33.333	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	Metamizole <7.5g	15	33.333	33.333	33.333
	Metamizole >7.5g	21	46.667	46.667	80.000
	Piritramide 22.5mg	9	20.000	20.000	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Late mobilization	**RRRM**	**LRM**	**Total**
Metamizole <7.5g	9	15	24
Metamizole >7.5g	21	21	42
Piritramide 22.5mg	15	9	24
Total	45	45	90

Chi-Squared Tests

	Value	df	p
X^2	3.000	2	0.223
N	90		

Table 9: *Frequency table for length of hospital stay in both groups with contingency table and chi-squared result for both groups*

Frequencies for hosp. stay

Group	Hosp. stay	Frequency	Percent	Valid Percent	Cumulative Percent
Rutkow-Robbins	1	24	53.333	53.333	53.333
	2	18	40.000	40.000	93.333
	3	3	6.667	6.667	100.000
	Missing	0	0.000		
	Total	45	100.000		
Lichtenstein	1	34	75.556	75.556	75.556
	2	9	20.000	20.000	95.556
	3	2	4.444	4.444	100.000
	Missing	0	0.000		
	Total	45	100.000		

Contingency Tables

	Group		
Hosp. stay	**RRRM**	**LRM**	**Total**
1	24	34	58
2	18	9	27
3	3	2	5
Total	45	45	90

group were hospitalised for 1, 2, and for 4 d compared to 24 (53.3%), 18 (40%), and three patients (6.7%) in the RRRM group for 1, 2, and 3 d, respectively (Table 9). There was no statistically significant difference in the frequency of postoperative complications, such as urine retention, bleeding in the first 24 h, bleeding > 24 h after the procedure, scrotal hematoma, wound and implant infections, mobility up to 24 h after the procedure, postoperative pain, the amount of analgesics consumed, testicular atrophy, length of hospitalisation, and hernia recurrence during the 24-month follow-up period, between the LRM and RRRM groups ($P > 0.005$). There was a noted difference in the BMI between the two groups. The RRRM with mesh and plug was shown to have no advantages compared to the LRM with mesh only with respect to the incidence of postoperative complications.

DISCUSSION

A low recurrence rate, a lack of pain, and a low frequency of postoperative complications are indicative of a successful hernia repair. Historically, hernias have been repaired using a tension method, which has been shown to be ineffective. Currently, all hernia repairs are performed using various tension-free methods. The most advanced method is a posterior repair done laparoscopically or robotically but the most common repair involves an open anterior repair method (1). The LRM, named after an American surgeon (Irving L. Lichtenstein), was developed in 1986 and soon became the standard for inguinal hernia repairs because of the lower recurrence rate. The LRM involves placing a mesh between the inguinal region and the aponeurosis of the obliquus externus muscle and eliminates the need for tension sutures (2). Various other tension-free

methods were subsequently developed. The RRRM is similar to the LRM but has the added element of placing and suturing a mesh plug into the actual defect, which adds an extra layer of reinforcement to prevent recurrence (3). In our retrospective study the incidence of postoperative outcomes was compared between the two types of hernia repair methods. Prior studies comparing these specific hernia repair methods are limited but concluded there was no difference between the methods in terms of outcomes but the operative time for the RRRM was less than the LRM (8, 9). A similar result was reported by Singh et al. (10) with no difference in outcomes but a shorter operative time in favour of RRRM. Meta-analyses have also shown no significant differences between the LRM and RRRM (11-13). The current study showed no difference in the postoperative outcomes but also revealed no significant difference in the length of the operation, and in fact showed an overall shorter time in the LRM group (Table 1, Figure 4). The duration of hospitalisation was comparable in both groups in the current study as well as other studies (Table 9) (14-16). The most important postoperative complication after an open anterior mesh repair is chronic pain. The occurrence rate is between 1% and 31% and has a direct impact on the quality of life. The main causes of persistent pain following a hernioplasty are mechanical triggers (specifically, trauma to the surrounding nerves and internal scarring) (1, 10, 16, 17). The severity of chronic pain after a hernioplasty varies between studies. None of the patients in the current study complained of chronic pain during follow-up evaluations. In fact, there was no significant difference in pain and usage of analgesics postoperatively but there was a trend in favour of LRM (Table 7). Early mobilisation was another parameter evaluated in the current study as an indicator of good hernia repair. No significant differences existed between the two groups with respect to early and late mobilisation (Table 8). Several other parameters were noted in the current study, including urinary retention, early and late bleeding, wound and implant infections or seromas, scrotal hematomas, and testicular atrophy but no significant differences were detected (Tables 3-6). It is also worth noting that there were no hernia recurrences during the follow-up period.

Antibiotic prophylaxis was shown to be an effective method in preventing surgical site infections and was used in all patients in the current study (19).

The current literature shows that a laparoscopic approach is superior to the LRM and RRRM. However, an open anterior repair, such as the LRM, is expected to retain a role in hernia treatment because the LRM is a low-cost and simpler procedure that does not require special equipment and has a shorter learning period with the added bonus of being able to be performed using local and/or regional anaesthesia (20-22). Some studies have shown an advantage for the RRRM with respect to operative and learning times. Indeed, the RRRM is simpler because less dissection is required compared to the LRM but this was not noted in the current study. However, this specific parameter cannot be assessed because all of the operations were performed by the same surgeon (4, 9, 17).

Various postoperative outcomes were compared between the LRM and RRRM hernioplasties in the current study. As reported by other researchers, no significant differences were detected between the LRM and RRRM hernioplasties. Therefore, no advantages with respect to postoperative outcomes were apparent between the two hernia repair methods. Because both hernia repair methods offer similar results, a slight advantage is given to the LRM because the LRM is the more cost-effective method. As methods for hernioplasty evolve, so do the techniques. The LRM has also evolved over time with new modifications and recommendations (23, 24).

CONCLUSION

To summarize, the current study compared the postoperative outcomes in male patients with unilateral inguinal hernias who underwent an elective LRM or RRRM hernioplasty. Both hernia repair methods were equally effective. Studies comparing these two hernia repair methods are limited with most of the studies reporting no significant differences between the two methods. The current study also showed no significant difference in postoperative

outcomes and recurrence rates between the LRM and RRRM. We conclude that both methods are safe and effective with a possible slight advantage going to the LRM solely due to cost.

CONFLICT OF INTEREST

The author declares no conflicts of interest.

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Prikaz primera dveh sorojencev s pozno infantilno obliko metakromatske levkodistrofije

Two siblings with late infantile form of Metachromatic leukodystrophy – a case report

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Ključne besede:
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Izvleček

Metakromatska levkodistrofija je avtosomno recesivna nevrodegenerativna bolezen. V osnovi gre za pomanjkanje encima arylsulfatase A oz. njegove aktivnosti, zaradi česar pride do kopičenja sulfiranih glikosfingolipidov v beli možganovini. Glede na pojav kliničnih znakov ločimo pozno infantilno, juvenilno in odraslo obliko bolezni. Bolezen je bila do nedavnega neozdravljiva. V prispevku prikazujemo primer sestre in brata, ki imata pozno infantilno obliko bolezni. Pri deklici je bila diagnoza postavljena, ko je bila bolezen že v napredovali fazi, zato je bila usmerjena v program paliativne oskrbe. Pri mlajšem bratu je bila bolezen ugotovljena v predsimptomatski fazi. Uvedeno je bilo eksperimentalno genetsko zdravljenje, ki je znatno spremenilo potek bolezni.

Abstract

Metachromatic leukodystrophy is an autosomal recessive neurodegenerative disease. The underlying mechanism of disease is the lack of arylsulphatase A, which leads to the accumulation of sulphated glycosphingolipids. Three forms of disease are recognized based on clinical onset, including infantile, juvenile, and adult forms. Until recently, metachromatic leukodystrophy was untreatable. In this article, we present the cases of a sister and brother with the late infantile form. The female was diagnosed when the disease was at an advanced stage, and thus, was treated palliatively. Her younger brother was diagnosed at the presymptomatic stage and experimental genetic treatment was conducted, which markedly Improved the course of disease.

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INTRODUCTION

Metachromatic leukodystrophy (MLD) is an autosomal recessive neurodegenerative disease of the white matter. The main cause of MLD is a deficiency of arylsulfatase A (ARSA), which is essential for the hydrolysis of sulphated glycosphingolipids. The deficiency of ARSA activity results in the storage of the sulphated glycosphingolipids inside the neuronal white matter. Over the clinical course of the disease, signs of upper and lower motor neuron pathology are observed. The clinical manifestation begins with irritability, inability to walk, and hyperextension of the knees. Following the progression of the disease, cognitive decline, psychiatric signs, and myoclonic seizures appear. Progressive muscle wasting leads to evident hypotonia and deep tendon reflexes become diminished or absent. Ocular signs progress from nystagmus to optic atrophy. In the late infantile form of MLD, clinical manifestations appear between the 12th and 18th months (M) with swift progression to tetraparesis and death during the first decade of life. The clinical signs of the juvenile form of MLD appear before 20 years of age (Y), while in the adult form, clinical manifestations become evident after 20 Y (1). Until a few years ago, there was no effective treatment for MLD (2,3). A genetic treatment was introduced in the last decade, and appears promising, but must be initiated at the pre-symptomatic or early symptomatic stages (4-6). Thus, efforts toward early diagnostic measures including the improvement of neonatal screenings are essential (7,8).

CASE PRESENTATION

The case concerns a family of healthy, non-consanguineous parents and three children. The first-born child (June 2014) was a healthy female. The second-born child was also a female (February 2017) and the third-born child was a male (February 2019) who were both affected by the late infantile form of MLD.

The second-born female had normal motor and cognitive development during the first year of life; however, she suffered several respiratory infections with an obstructive pulmonary component. During the infections, her motor milestones halted or regressed. The parents reported that the child would refuse to stand on her feet or walk for approximately 2 weeks. The child began walking without support at 15 M. At 20 M she was examined by a developmental neurologist, who described normal motor and cognitive development, except axial hypotonia. One month later, she was hospitalised due to a simultaneous parainfluenza and Chlamydophila pneumoniae infection. At 22 M, she was hospitalized again due to influenza. After hospitalisation, she began to complain of leg pain and at 24 M she stopped walking, but was able to sit without support. At 25 M she was hospitalized at the University Paediatric Clinic of Ljubljana due to evident motor regression, generalised muscular weakness (including facial muscles), muscular pain, inability to be seated without support, and lacking deep tendon reflexes. A lumbal puncture revealed elevated protein. Electromyography (EMG) was consistent with a demyelinating process. A diagnosis of Guillaine-Barre syndrome was suggested and after the first therapy with intravenous immunoglobulins, the clinical signs improved, except for the muscular weakness in the legs. Despite intensive physiotherapy and repeated immunoglobulin treatment, progressive motor deterioration was noted. Further diagnostics were performed for neuro-degenerative diseases.

Magnetic resonance imaging (MRI) of the brain at 28 M revealed bilateral symmetrical alterations of the deep periventricular white matter and corpus callosum, as well as changes to the thickness of the cervical nerve roots. Low ARSA activity was found in the peripheral blood. Genetic testing at 30 M confirmed the diagnosis of MLD and due to the advanced clinical signs, the patient was referred to a palliative care program.

The disease progressed as expected. At 3 Y, the patient was unable to sit independently, but she could lift her head. Despite progressive motor decline, her cognitive development was good. At 3 Y and 2 M, oral feeding became impossible, and a nasogastric tube was inserted. At 3 Y and 6 M, her cognitive

and motor performance declined rapidly and she was able to communicate only with her eyes. Permanent salivation, painful muscle spasms, rigidity and grand mal epileptic episodes developed. Therapy with morphine, gabapentin, levetiracetam, clonidine, and glycopyrrolate was introduced to reduce the pain, seizures, salivation, and other symptoms. By 4 Y, the patient was completely bedridden, with almost no communication. A percutaneous gastrostomy was used to facilitate nutrition.

The patient required frequent upper respiratory tract aspirations, urinary bladder catheterisations, and regular therapy with lactulose or macrogol to maintain regular defecation. Regular physiotherapy once per week and an annual 2-week rehabilitation at a seaside children's rehabilitation centre are included in the palliative care to reduce the spasticity, pain, and respiratory difficulties. She suffers frequent respiratory and urinary infections, which are treated with oral antibiotics.

The male child was born at 40 weeks of gestational age after an uneventful, normal pregnancy. His early developmental milestones were normal. The boy was 8 M of age when his older sister was diagnosed with MLD and he underwent immediate biochemical and genetic testing. Low ARSA activity was detected in the peripheral blood (< 1 nmol/hr/mg). The same mutations as his older sister were found, confirming the MLD diagnosis. As the patient was in the pre-symptomatic stage of the disease, he was enrolled in a clinical study for the treatment of MLD with gene therapy. After the parents' written consent was obtained, his inclusion into the study began at the San Raffaele Hospital in Milano, Italy (4-6).

The patient's brain MRI was normal and electroencephalography (EEG) showed no pathological significance. Electromyography (EMG) registered normal sensory and motor conduction. The brainstem evoked auditory potentials with mild left side peripheral hearing loss (increased latency at high frequences 90-95 dB).

At 10 M the patient underwent transplantation of autologous cryopreserved bone marrow CD34+ cells transduced ex vivo with lentiviral vector encoding human ARSA cDNA after a conditioning regimen with intravenous Busulfan. After therapy, normal ARSA activity was noted in the peripheral blood and has remained within the normal range 4 years later. The patient has regular check-ups twice per year at the San Raffaele Hospital in Milano. His cognitive development is assessed regularly by a development neurologist and a psychologist. He pronounced his first words at 11 M, two-word sentences at 19 M, three-word sentences at 21 M, and sentences of six words or more at 26-27 M. He began crawling at 9 M, and began walking independently at 16 M. His motor development is impaired, with gait instability, hyperextensions of the knees, lower limb weakness, and mild spasticity, but he can walk, jump, run, and ride a bicycle. He attends regular physiotherapy two times per week and once per year he attends 2-week rehabilitation sessions with his sister in a children's rehabilitation centre near the seaside. At 5 Y, the patient attended a 4-week rehabilitation session in the national rehabilitation centre, which included physiotherapy, occupational therapy, and speech therapy. He is supported with ankle foot orthosis and is very communicative and curious, and his motor functions are improving.

The patient attends kindergarten with the support of a personal assistant due to his motor instability. In September 2025, he will begin attending school.

Discussion from the view of a primary care paediatrician

This study detailed two siblings with the same type of a rare neurodegenerative disease. The female's diagnosis was discovered based on progressive clinical manifestations of leukodystrophy. Due to the clinical signs of motor impairment, with brain, spine and peripheral nerve white matter degeneration, no adequate therapy could be provided.As the first rule of paediatric palliative care is to never stop treating the patient, we tried everything to help the family, and make the patient's life easier. Despite losing her ability to see, hear, and speak, the patient remains highly perceptive to touch and responds differently, depending on the person touching her. It is very important that the patient has access to medical care professionals who understand her needs, and have the confidence of the family (9-12).

The male patient was a healthy baby when the parents were informed of the female's diagnosis. A few weeks later, the boy received the same diagnosis. A new gene therapy for MLD was being conducted in the San Raffaele paediatric hospital in Milano, Italy. As the boy lacked clinical signs of MLD, the therapy was proposed to his parents with descriptions of its potential risks and benefits. They went through a process of doubt and fear of whether the therapy would provide a better life to their child or simply prolong the suffering. The fears and uncertainty regarding whether the therapy would be successful or not persisted through the boy's first years of life, and will probably last for his life. But his mental development appears excellent. Although he suffered motor impairment, he is a positive, healthy, and happy child.

The eldest child was approximately 4 Y when her younger sister became ill. Later, the brother travelled for treatment, and their mother needed to remain with him. The grandparents supported the family by taking care of the second child. During that time, psychological support was offered to the eldest child and she is currently attending primary school, receiving excellent grades, and is doing well in sports. The family is well organised regarding the treatment of the younger children, while attempting to live their family life as normal as possible. The support of the extended family (e.g., the children's grandparents) is precious.

CONCLUSIONS

The late infantile form of MLD is a neurodegenerative disease with a swift progression. When left untreated, it leads to death within the first decade of life. There have been no efficient therapies for MLD until recently (2,3). Gene therapy with transduced autologous haematopoietic stem cells appears effective, but must be initiated during the pre-symptomatic phase (4-6); and therefore, an early diagnosis is crucial. The implementation of newborn screening along with available gene therapy will provide improved lives for affected children and their families (7, 8). Paediatric palliative care should be a standard practice in paediatric primary care for all children with untreatable and terminal diseases (9-12).

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Fitz-Hugh–Curtisov sindrom pri adolescentki: zapoznela diagnoza subtilne klinične slike

Fitz-Hugh–Curtis Syndrome in an Adolescent: Delayed Diagnosis due to a Subtle Presentation

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Ključne besede:
Fitz-Hugh-Curtisov sindrom, pelvična vnetna bolezen, magnetna resonanca, *Chlamydia trachomatis*, bolečina v ramenu

Key words:
Fitz-Hugh-Curtis syndrome, PID, MRI, *Chlamydia* infections, Shoulder pain

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Izvleček

Namen: *Predstaviti diagnostični pomen magnetnoresonančnega slikanja (MRI) pri prepoznavanju Fitz-Hugh–Curtisovega sindroma (FHCS) pri mladostni bolnici s subtilno klinično sliko in poudariti pomen pravočasnega razmisleka o medenični vnetni bolezni (PID) v tej starostni skupini.*
Metode: *Sedemnajstletna športno aktivna bolnica je bila sprejeta zaradi bolečine v desnem zgornjem kvadrantu trebuha in bolečine v desni rami. Začetna obravnava z laboratorijskimi preiskavami, ultrazvokom (UZ) in računalniško tomografijo (CT) ni pojasnila etiologije težav. Zaradi vztrajajočih simptomov, je bil opravljen MRI abdomna in male medenice.*
Rezultati: *MRI je razkril obojestranski piosalpinks z restrikticijo difuzije, prosto tekočino v mali medenici in subhepatičnem prostoru ter tanke adhezije med jetrno kapsulo in peritonejem, skladne s Fitz-Hugh–Curt-*

Abstract

Purpose: *To emphasize the diagnostic role of magnetic resonance imaging (MRI) in identifying Fitz-Hugh–Curtis syndrome (FHCS) in an adolescent patient with a subtle clinical presentation.*
Methods: *A 17-year-old female presented with acute onset right upper quadrant and right shoulder pain. The initial diagnostic workup, including ultrasound (US), computed tomography (CT), and laboratory testing was inconclusive. Due to persistent symptoms and elevated inflammatory markers, MRI of the abdomen and pelvis was subsequently performed.*
Results: *MRI demonstrated a bilateral pyosalpinx with restricted diffusion, free fluid in the pelvis and subhepatic region, and linear adhesions between the hepatic capsule and peritoneum, which was consistent with pelvic inflammatory dis-*

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isovim sindromom kot zapletom PID. Mikrobiološke preiskave so potrdile okužbo s Chlamydia trachomatis*. Po ustreznem antibiotičnem zdravljenju je prišlo do popolnega kliničnega izboljšanja.*

Sklep: *Fitz-Hugh–Curtisov sindrom je redek, vendar pomemben zaplet PID, ki se lahko kaže z nespecifično bolečino v desnem zgornjem kvadrantu in preneseno bolečino v rami. MRI predstavlja metodo izbora za potrditev PID in njenih zapletov, saj omogoča visoko diagnostično natančnost brez izpostavljenosti sevanju, kar je posebej pomembno pri mladostni populaciji.*

ease (PID) complicated by FHCS. Microbiologic analysis confirmed a Chlamydia trachomatis *infection. The patient received targeted antibiotic therapy with clinical improvement.*

Conclusion: *FHCS represents a rare but important complication of PID that may present with non-specific or extra-pelvic symptoms. MRI is the modality of choice for confirming PID-related complications and perihepatic inflammation, offering high sensitivity and the advantage of radiation-free imaging, which is especially relevant in the adolescent population.*

INTRODUCTION

Fitz-Hugh–Curtis syndrome (FHCS) or perihepatitis is a chronic inflammatory condition of the liver capsule associated with genital tract infection and represents a complication of pelvic inflammatory disease (PID), primarily affecting women of reproductive age (1,2). FHCS is characterized by perihepatic adhesions and right upper quadrant pain caused by fibrous connections between the anterior liver surface and abdominal wall (1). The pain often worsens with movement or deep breathing and may mimic acute abdominal conditions, such as cholecystitis, pyelonephritis, pneumonia, or appendicitis (3,4). Shoulder pain may also occur as referred pain due to diaphragmatic irritation and typically worsens with deep inspiration, which should raise suspicion of a subdiaphragmatic or hepatic origin (4). Importantly, FHCS can occur even in the absence of overt PID symptoms, particularly in young women, in whom delayed diagnosis can increase the risk of tubal damage and subsequent infertility (4).

FHCS represents one end of the PID spectrum. PID refers to infection and inflammation of the female upper genital tract, ranging in severity from mild pelvic inflammation to severe complications, such as tubo-ovarian abscess (TOA), pyosalpinx, or oophoritis. FHCS arises as one of these potential chronic complications. Because the presentation of FHCS is often non-specific, maintaining a high level of clinical suspicion is essential. PID primarily affects women of reproductive age and is rare in postmenopausal or prepubertal women (5).

Chlamydia trachomatis is most frequently implicated among the pathogens associated with FHCS. This bacterium is a leading cause of sexually transmitted infections worldwide and can induce persistent genital tract inflammation, contributing to infertility, chronic pelvic pain, and ectopic pregnancy if left untreated in a timely fashion (6).

IMAGING FEATURES OF PID AND FHCS

While ultrasound (US) and computed tomography (CT) are often first-line modalities in the evaluation of PID and PID complications (peritonitis, abscess formation, and infertility), magnetic resonance imaging (MRI) has substantially higher sensitivity (up to 95%) compared to contrast-enhanced CT (79%), although CT has slightly higher specificity (99% vs. 89%). MRI sensitivity is increased to nearly 98% while maintaining high specificity with incorporation of diffusion-weighted imaging (DWI) (7,8). CT imaging findings in PID range from adnexal mass formation to thickening and contrast enhancement of the fallopian tubes, loss of clear boundaries between the adnexa and uterus, pelvic fat stranding, and thickening of the uterosacral ligaments. Pathologic changes associated with PID include cervicitis, endometritis and uterine

empyema (pyometra), oophoritis, TOA, peritonitis, and salpingitis with tubal empyema (pyosalpinx). Salpingitis represents the earliest stage of PID and involves inflammation of the fallopian tubes, typically due to sexually transmitted infections. If untreated, salpingitis can progress to tubal empyema (pyosalpinx) with purulence. Imaging findings are variable and may range from mild tubal wall thickening (≥ 5 mm) and surrounding fat stranding-to-fluid-filled, thick-walled, enhancing tubes consistent with empyema. Chronic cases may lead to adhesions, tubal distortion, and infertility (8). Pelvic adhesions may appear on MRI as thin or thick linear bands or sheet-like structures connecting pelvic organs or peritoneal surfaces. These fibrous or vascularized adhesions can occasionally show mild post-contrast enhancement and may distort adjacent visceral contours (15). A dilated tubular fallopian structure exhibiting heterogeneous internal signal intensity on conventional MRI sequences with wall thickening and contrast enhancement, especially when DWI shows restricted diffusion in the walls and purulent contents, is highly suggestive of a pyosalpinx or an associated TOA (13).A TOA is a severe, complex complication of PID that is characterized by accumulation of pus in the fallopian tubes and ovaries. Imaging findings typically show septated, multilocular cystic adnexal masses with thick, enhancing walls (8). TOAs typically appear on MRI as multiloculated cystic pelvic masses with heterogeneous high signal intensity on T2-weighted images and low signal intensity on T1-weighted images, often demonstrating the penumbra sign (a hyperintense rim on T1), according to a recent review by Ferenc and Popić (2024). Post-contrast images usually show rim and septal enhancement, while DWI reveals hyperintense cystic components with restricted diffusion and low apparent diffusion coefficient values. TOAs are more frequently associated with tubal dilatation, adjacent organ involvement, and smaller overall size compared to malignant adnexal masses (12). PID can coexist with inflammation of the endometrium or ovaries, may progress to involve the peritoneum, and can occasionally extend along the paracolic gutters to the hepatic surface, resulting in FHCS (13).

Due to non-specific symptoms, such as pleuritic right upper quadrant pain and referred shoulder pain, diagnosis of FHCS can be challenging (pelvic pain, cervical motion tenderness, and vaginal discharge are often absent). Referred shoulder pain is a common clinical feature of FHCS, representing pain perceived in the shoulder region due to irritation of the diaphragm and phrenic nerve by hepatic capsular inflammation (16). CT findings include increased hepatic capsular enhancement during the arterial phase, capsular thickening of the liver, fluid and fat stranding extending from the pelvis to the right upper quadrant through the paracolic gutter, gallbladder wall thickening, and loculated perihepatic fluid. Capsular enhancement of the liver (Glisson's capsule) is the hallmark finding of perihepatitis. The enhancement is most evident on early post-contrast images using a biphasic protocol with acquisitions at 35–40 s (arterial phase) and ~70 s (portal phase). Review of images with narrow window settings improves capsule–parenchyma contrast and helps detect subtle capsular thickening or enhancement. US findings are non-specific and mainly include a thickened hepatic capsule and ascites, although the US findings may be interpreted as normal. These findings can also be present in perihepatitis due to other causes, such as perforated cholecystitis or hepatic abscess, tuberculous peritonitis, and peritoneal carcinomatosis (8,9,14). MRI findings in perihepatitis are infrequently described in the literature. Specifically, MRI revealed linear perihepatic and subcapsular contrast enhancement corresponding to capsular inflammation, mild perihepatic fluid in Morrison's pouch, and increased T2 signal along the hepatic surface and subcapsular regions in two reported cases (a 34-year-old woman and a 17-year-old girl), consistent with edema and early inflammatory changes. In the younger patient, the subcapsular T2 hyperintensity was interpreted as a small area of combined ascites and edema (10,11).

CASE PRESENTATION

A 17-year-old otherwise physically active girl presented to the Pediatric Emergency Department in November 2024 due to pain below the right costal margin, which intensified with inspiration. She reported that the symptoms had started with right shoulder pain 10 days earlier. She had no history of chronic diseases. The epidemiologic history included travel to Asia 1 month before presentation.

On physical examination, tenderness was noted in the right lower quadrant with positive McBurney's and Rovsing's signs. Laboratory test findings showed an elevated C-reactive protein (CRP) (50 mg/L) and a significantly elevated D-dimer (1246 µg/L). A chest X-ray excluded pneumonia and an abdominal US excluded acute appendicitis (the appendix had normal diameter (< 6 mm)) without visible structural changes in the surrounding fat tissue but free fluid was noted in the pouch of Douglas. The patient was admitted for further diagnostics and observation due to a suspected intra-abdominal inflammatory process (pain, elevated D-dimer, and moderately increased CRP; Figure 1). During the 3-week hospitalization, an abdominal US was repeated twice, again showing free fluid in the true pelvis, the appendix without signs of inflammation, and B-lines noted along the liver surface under the right hemidiaphragm. Doppler US of the veins was performed due to an elevated D-dimer, excluding a deep vein thrombosis. Because of pain in the right shoulder, US and MRI of the right shoulder were performed, showing no significant abnormalities except for mild supraspinatus tendinosis.

Due to persistent inspiratory pain in the right upper quadrant and an elevated D-dimer (which increased during hospitalization to 3529 µg/L), CT angiography of the pulmonary arteries was performed, which excluded a pulmonary embolism. Because the pain persisted, a pelvic US and MRI were performed. The pelvic US was unremarkable but the MRI of the pelvis revealed free fluid in the true pelvis that was greater than physiologically expected. MRI diagnostics

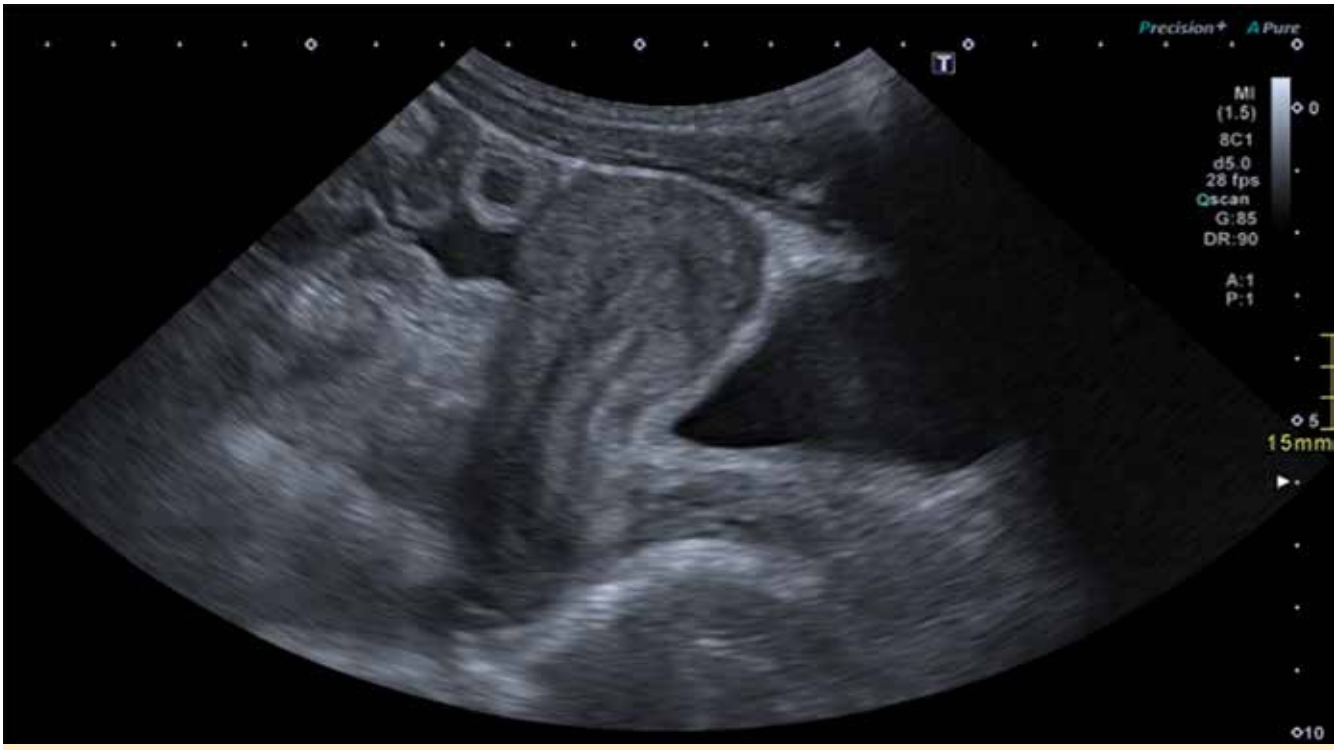

Figure 1. *The first ultrasound performed in November 2024. The transverse view of the pelvis posterior to the uterus (anterior to the urinary bladder is visualized, filled with hypoechoic urine) shows a collection of free fluid in the pelvic cavity, the amount of which is greater than physiologically expected.*

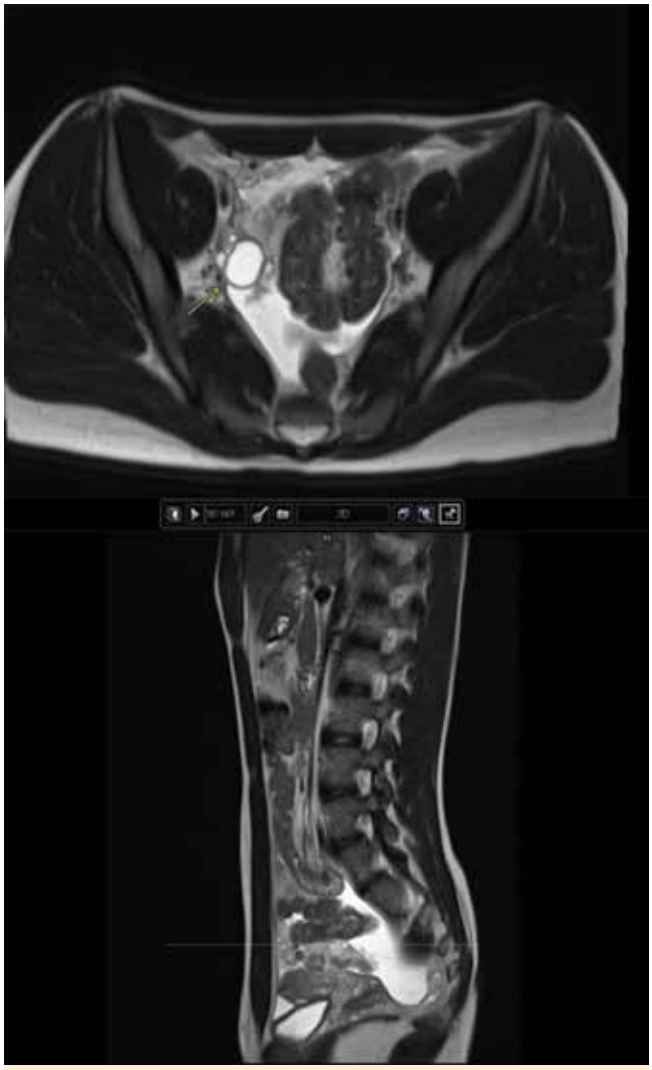

Figure 2. *The first MRI of the abdomen and pelvis performed in November during the initial hospitalization (T2-weighted axial and coronal planes) demonstrates T2-hyperintense free fluid in the pelvic cavity, greater in amount than physiologically expected. The arrow on the axial image indicates a hyperintense lesion on the right, corresponding to an ovarian cyst.*

were performed according to the protocol shown in Table 1. A positron emission tomography-CT did not show scintigraphic evidence of acute inflammation or malignancy. The patient was discharged to home after the inflammatory marker and D-dimer levels decreased althoughthe cause of the symptoms remained unclear during the hospitalization. She was referred for follow-up care in the Pediatric Gastroenterology Clinic (Figure 2).

No abnormalities were present on clinical examination at the follow-up appointment in January 2025(the white blood cell count, CRP level, and renal and liver function tests were normal), but the D-dimer level remained elevated (3626 µg/L). Consultations with a hematologist and pediatric rheumatologist were scheduled.

During the hematology consultation in early February 2025 the patient reported a weight loss of 3 kg since the previous hospitalization. She did not describe any pain or loss of appetite. The physical examination was unremarkable. Laboratory test findings again showed a significantly elevated D-dimer (15924 µg/L), therefore she was hospitalized for further diagnostic evaluation.

The second hospitalization in February 2025 occurred 58 days after discharge from the first hospitalization. The patient was hospitalized for 1 week, during which magnetic resonance angiography of the thoracic and abdominal aorta was performed due to suspicion of vasculitis. The arterial origins were normal but only a short segment of the celiac trunk was visualized on T2 sequences and the distal course was not seen, raising suspicion for median arcuate ligament syndrome. A follow-up abdominal US and Doppler assessment of the arteries showed a slightly narrowed origin of the celiac trunk with velocity variation during inspiration and expiration, which were consistent with median arcuate ligament syndrome.

Free fluid was again noted on US in the pelvis and subhepatic and perisplenic regions. Both ovaries were enlarged with tubular, hypoechoic, peripherally vascularized structures (i.e., the fallopian tubes), for which a gynecologic evaluation was recommended. A gynecologic US showed enlarged ovaries with areas of denser content, raising suspicion for TOAs. Free fluid in the pouch of Douglas and adhesions were also noted. During the gynecologic consultation cervical swabs and serologic testing for *C. trachomatis* antibodies were obtained, and an MRI of the abdomen and pelvis was repeated (Figure 3).

On abdominal MRI the liver was at the upper limit of normal size with a T2-hyperintense line

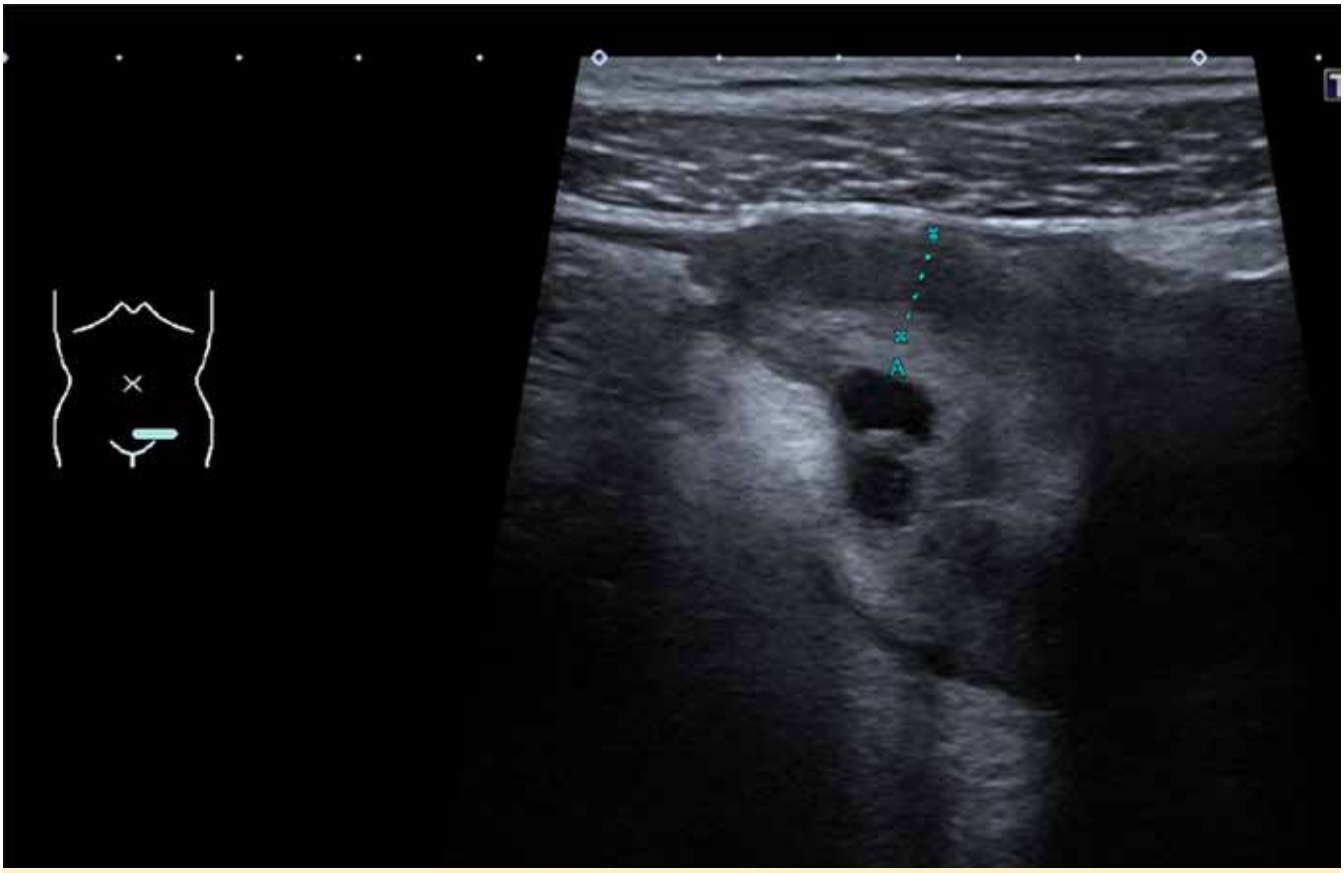

Figure 3. *During the second hospitalization, a tubular structure was visualized adjacent to both ovaries on ultrasound (the image above shows the left side). The structure was filled with hypoechoic content, a thickened wall, and peripheral vascularization. This finding most likely represented a fallopian tube distended with fluid, suspicious for salpingitis or pyosalpinx.*

representing subhepatic free fluid. Several thin linear bands connecting the liver to the peritoneal surface were visualized in this area, representing adhesions. Both fallopian tubes were dilated and filled with T2-hyperintense content, which was slightly lower in intensity compared to the T2 signal of ovarian cysts. Restricted diffusion (hyperintense signal on DWI and hypointense signal on the apparent diffusion coefficient map) and thickened tubal walls were noted, consistent with salpingitis or a pyosalpinx. Free fluid in the true pelvis was noted again, along with thin linear bands corresponding to fibrin strands and fibrosis (iso- to mildly hypo-intense signal on T1 and low signal on T2 compared with normal myometrium) in theposterior vaginal fornix and retrocervical portion of the uterus. A vaginal polymerase chain reaction confirmed C. trachomatis. *Ureaplasma parvum* was also detected, likely representing colonization (Figures 4–6).

The patient was treated by the gynecology team with ceftriaxone (500 mg intramuscularly once) and dual oral antibiotic therapy (doxycycline (100 mg 1 tablet every 12 h for 10 d) and metronidazole (400 mg 1 tablet every 12 d for 14 d)). Follow-up care was arranged in the Gynecology Clinic.

Hematologic testing showed that the patient is a carrier of the Factor V Leiden mutation, which increases the risk of thromboembolic events. She received counseling regarding lifestyle modifications to include maintaining adequate hydration, avoiding prolonged sitting, avoiding caffeine, alcohol, and smoking, maintaining normal body weight, and being cautious with the use of oral contraceptives. She was

advised to use prophylactic low-molecular-weight heparin in case of fractures or prolonged flights and to have regular follow-up in the Anticoagulation Clinic.

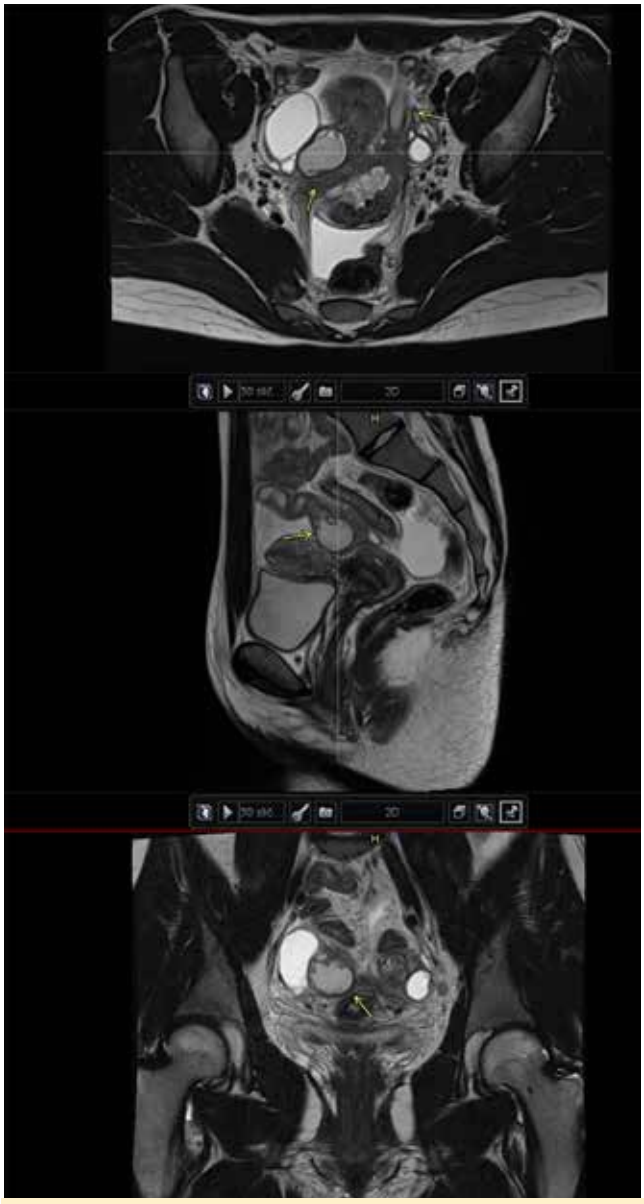

Figure 4. *MRI of the pelvis during the second hospitalization in February (T2-weighted axial, sagittal, and coronal planes) revealed bilateral tubular adnexal formations filled with T2-hyperintense content, slightly less intense than that of the adjacent ovarian cysts. These findings correspond to dilated fallopian tubes with thickened walls, consistent with a pyosalpinx. Additional T2-hyperintense free fluid was present in the pouch of Douglas, greater in amount than physiologically expected.*

An abdominal US in late February 2025 showed partial regression of ascites in the true pelvis with persistent heterogeneous changes in the left fallopian tube.

At the end of June 2025 (approximately 6 months after the diagnosis of PID), follow-up in the Pediatric Clinic revealed no abnormalities with normal clinical findings and laboratory parameters.

A follow-up US in August 2025 showed a small amount of free fluid in the small pelvis (up to 15 mL), which was within physiologic expected limits (14 d after the last menstruation in the periovulatory period). No records of gynecologic follow-up examinations were received and no gynecology records were found in the electronic medical record system or from other institutions.

DISCUSSION

This case illustrates the diagnostic value of cross-sectional imaging (especially MRI) in identifying FHCS in an adolescent patient with an subtle presentation. FHCS is a rare complication of PID that is most often caused by *C. trachomatis* and characterized by perihepatic inflammation and adhesions. While FHCS typically presents with right upper quadrant pain, referred shoulder pain due to diaphragmatic irritation, as occurred in this case, can delay diagnosis.

Because of her athletic background, early evaluation was directed toward musculoskeletal etiologies, a focus that contributed to the diagnostic delay. US, while useful for excluding appendicitis and identifying free pelvic fluid, lacked specificity for the extent of pelvic and perihepatic involvement. As described and suggested by Moon et al., US findings (thickened hepatic capsule and ascites in women and adolescent girls of reproductive age) should raise suspicion for PID and the possibility of FHCS when supported by clinical findings (9). A CT scan may demonstrate hepatic capsular enhancement and fluid tracking along the paracolic gutters but in this adolescent patient MRI was advantageous in terms of tissue characterization without ionizing radiation. MRI was crucial in establishing the diagnosis and has been shown to be more effective than both US and CT (7,8). Findings, including bilateral pyosalpinx

with diffusion restriction, fallopian tube distention with T2-hypointense content, pelvic ascites, and subhepatic adhesions, were critical in confirming

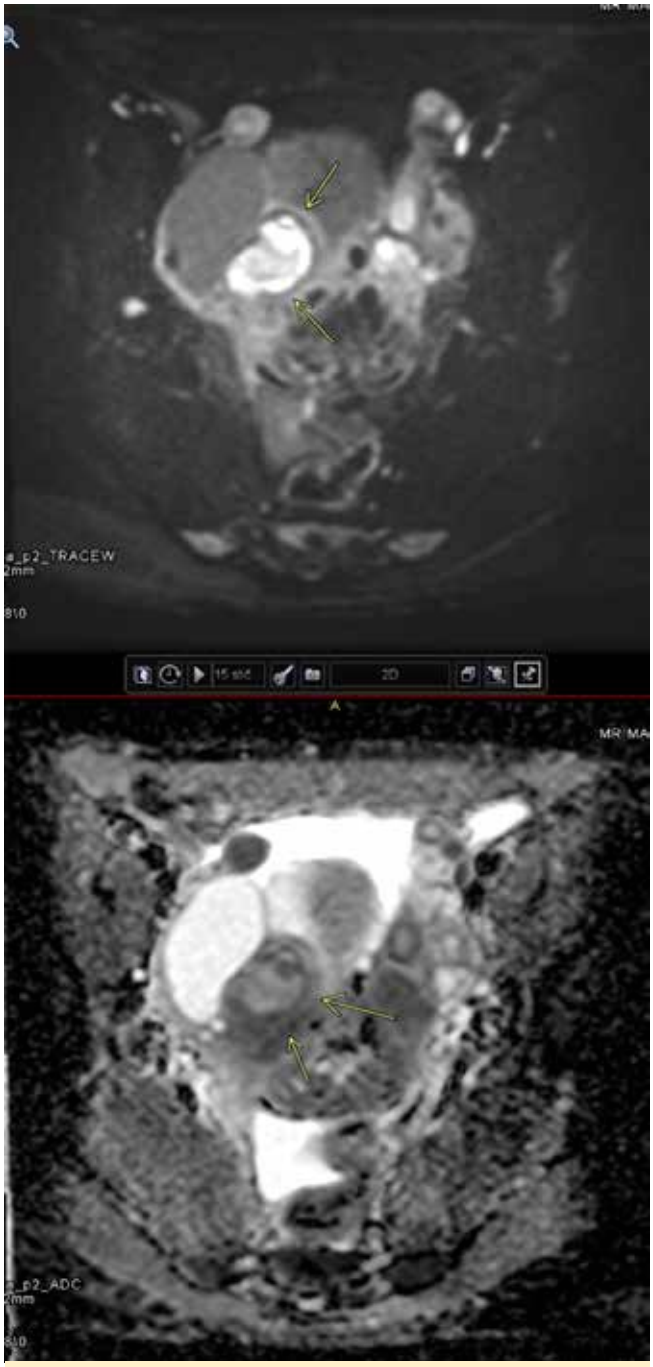

Figure 5. *Diffusion-weighted MRI of the pelvis (axial views) and the corresponding ADC map show marked hyperintensity on DWI and low signal on ADC within the fallopian tube regions (arrows), consistent with restricted diffusion, supporting the diagnosis of an active inflammatory or purulent process.*

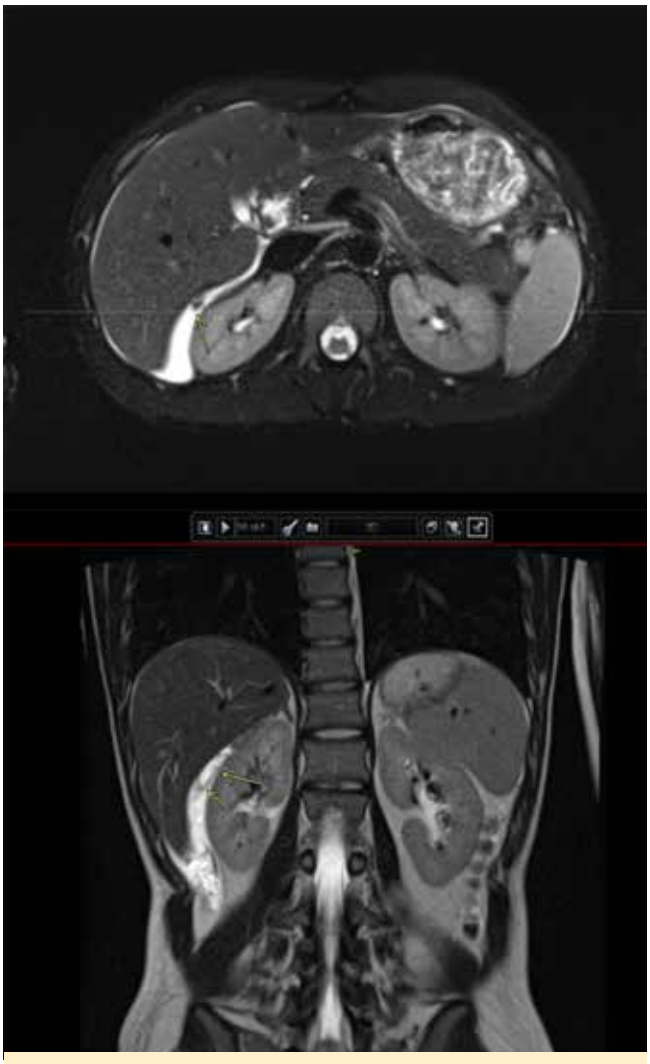

Figure 6. *MRI of the upper abdomen (T2-weighted axial and coronal planes) demonstrates a layer of T2 hyperintense fluid adjacent to the liver, consistent with free intraperitoneal fluid (a thin layer is also visible along the spleen). Within the perihepatic fluid, thin T2 hypointense linear bands are observed, representing adhesions.*

FHCS. As described in a case report by Shibuya et al. and in a multi-case study by Kubo et al., MRI following US (which may be normal) is the imaging modality of choice when FHCS is suspected because MRI avoids ionizing radiation and provides better soft-tissue characterization than CT, especially in the adolescent population (10,11). MRI is particularly valuable in this population due to high sensitivity for PID-related complications and the ability to

Table 1: *MRI Protocol Summary: Upper Abdomen and Pelvis*

Parameter	Description
Scanner	1.5 Tesla
Coil Used	Phased-array body/pelvic coil
Purpose	Optimize signal-to-noise ratio
T1-Weighted Sequences	VIBE (opposed-phase, in-phase, water-only, and fat-only) sequences
T2-Weighted Sequences	HASTE sequences in axial and coronal planes with and without fat suppression
Diffusion-Weighted Imaging (DWI)	b-values: 0, 500, 800 s/mm²; ADC maps for quantitative diffusion assessment
Post-Contrast Imaging	T1-weighted VIBE sequences in axial and coronal planes after intravenous gadolinium administration
Dynamic Contrast Phases	25 s (late arterial/early venous), 60 s (portal venous, with/without subtraction), 2 min (delayed), 3 min (coronal delayed)
Slice Thickness	Upper abdomen: 4 mm (2.6 mm for contrast-enhanced sequences); Pelvis: 3 mm (2.6 mm for contrast-enhanced sequences)
Interslice Gap	Minimal
Special Notes	Protocol applies to both upper abdomen and pelvis; includes ADC maps for diffusion assessment

depict subtle perihepatic changes. Individuals with sexually transmitted infections are advised to receive medical counseling, which emphasizes the importance of promoting sexual health and preventing sexually transmitted diseases through awareness, risk-reduction strategies, empathic and non-judgmental counseling, and high-intensity behavioral interventions tailored to patient risk (17).

CONCLUSION

FHCS should be considered in adolescent and young adult women presenting with right upper quadrant or referred shoulder pain, especially when US demonstrates free pelvic fluid or hepatic capsular thickening despite minimal pelvic symptoms. MRI can further characterize PID (e.g., pyosalpinx) and perihepatic involvement without ionizing radiation, thereby supporting diagnostic clarification and timely clinical management.

CONFLICT OF INTEREST

No conflict of interest to declare.

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ETHIC APPROVAL SENTENCE

Since the patient turned 18 during the course of medical management, a consent form was obtained from her authorizing the use of anonymized medical data for presentation purposes.

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