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Nanotechnology-Driven Strategies to Combat Antimicrobial Resistance in the One Health Era

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ABSTRACT

Antimicrobial resistance (AMR) has emerged as one of the greatest global public health threats, compromising the effectiveness of antibiotics and jeopardizing advances in modern medicine. The extensive use and misuse of antimicrobial agents in human medicine, veterinary practice, agriculture, and aquaculture have accelerated the emergence and spread of multidrug-resistant microorganisms. Because resistant pathogens, antibiotic residues, and resistance genes continuously circulate among humans, animals, and the environment, AMR is now recognized as a complex One Health challenge requiring coordinated, multidisciplinary solutions. However, conventional strategies, including the development of new antibiotics and antimicrobial stewardship programs, are increasingly constrained by rapid microbial adaptation, biofilm formation, delayed diagnosis, and the declining antibiotic discovery pipeline. Nanotechnology has emerged as a promising interdisciplinary platform capable of addressing several limitations of conventional antimicrobial approaches. Nanomaterials possess intrinsic broad-spectrum antimicrobial activity, enhance targeted drug delivery, improve biofilm eradication, and enable rapid pathogen detection through advanced nanodiagnostic systems. Furthermore, integrating nanotechnology with artificial intelligence, machine learning, and smart biosensing technologies is accelerating the development of precision antimicrobial therapies and real-time AMR surveillance systems. This review discusses the AMR crisis from a One Health perspective and summarizes recent advances in antimicrobial nanomaterials, nanodrug delivery systems, antibiofilm strategies, and nanodiagnostics. Current translational challenges and future perspectives are also highlighted, emphasizing the potential of nanotechnology to support sustainable AMR management across interconnected human, animal, and environmental sectors.

Keywords: Antimicrobial resistance, artificial intelligence, biofilms, nanodiagnostics, nanodrug delivery, nanoparticles, nanotechnology, One Health.

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INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most serious threats to global public health, modern medicine, food security, and economic stability.[1,2] The extraordinary success of antibiotics during the twentieth century revolutionized healthcare and dramatically reduced

mortality from infectious diseases.[3] However, decades of excessive and inappropriate antimicrobial use in human medicine, veterinary practice, agriculture, and aquaculture have accelerated the emergence of resistant microorganisms capable of surviving even the most potent antimicrobial therapies.[4] Today, infections that were once considered easily treatable are becoming increasingly difficult and time-consuming to treat and, in some cases, impossible to cure.

The consequences of AMR extend far beyond individual patient outcomes. Resistant pathogens threaten essential medical procedures, including organ transplantation, cancer chemotherapy, neonatal care, and major surgery, all of which rely heavily on effective infection control.[2] According to global estimates, AMR already contributes to millions of deaths annually and may result in approximately 10 million deaths per year by 2050 unless urgent interventions are implemented.[1] In addition to its devastating health burden, AMR imposes enormous economic costs through prolonged hospitalizations, increased healthcare expenditures, productivity losses, and strain on healthcare infrastructure worldwide.[1,2]

One of the greatest challenges in addressing AMR is that resistance does not emerge or spread within a single, isolated environment. Instead, resistant microorganisms continuously circulate among humans, animals, food products, and environmental reservoirs through highly interconnected transmission pathways.[5] Antibiotics used in livestock production may select for resistant bacteria that enter the food chain, whereas hospital effluents, pharmaceutical waste, and agricultural runoff introduce antimicrobial residues and resistance genes into aquatic and soil ecosystems.[6] Environmental microorganisms further amplify the dissemination of resistance through horizontal gene transfer mechanisms mediated by plasmids, transposons, and other mobile genetic elements. Consequently, AMR has evolved from a purely clinical problem into a complex ecological and global health crisis.

This interconnected pattern of resistance dissemination has emphasized the importance of the One Health concept, which recognizes the close relationships among human, animal, and environmental health. The One Health framework underscores that effective AMR mitigation cannot rely solely on hospital-based antibiotic stewardship programs or the discovery of new antimicrobial drugs. Instead, sustainable control strategies require coordinated, multidisciplinary collaboration among clinicians, microbiologists, veterinarians, environmental scientists, policymakers, and public health authorities. Without integrated surveillance systems and cross-sectoral interventions, resistant pathogens and resistance genes will continue to circulate across ecosystems regardless of local containment efforts.

Despite decades of antimicrobial development, conventional antibiotics are increasingly ineffective against multidrug-resistant pathogens. Microorganisms possess remarkable adaptive capabilities that enable the rapid evolution of resistance mechanisms, including enzymatic antibiotic degradation, target modification, altered membrane permeability, active efflux systems, and biofilm formation.[7] Meanwhile, the antibiotic discovery pipeline has slowed dramatically because of scientific challenges, high development costs, and limited economic incentives for pharmaceutical companies.[3] As resistance evolves more rapidly than new antibiotics are introduced, the effectiveness of conventional antimicrobial therapies continues to decline.

In response to these growing limitations, nanotechnology has emerged as one of the most promising interdisciplinary approaches to combating AMR. Nanomaterials possess unique physicochemical properties, including nanoscale dimensions, high surface-area-to-volume ratios, tunable surface chemistry, and multifunctionality, that enable novel antimicrobial applications.[8] Unlike traditional antibiotics, which often target a single cellular pathway, nanoparticles can attack microorganisms through multiple simultaneous mechanisms, such as reactive oxygen species generation, membrane disruption, protein dysfunction, and nucleic acid damage, thereby reducing the likelihood of resistance development.[9]

Importantly, nanotechnology offers solutions that extend across all One Health domains. In human medicine, nano-enabled systems improve targeted drug delivery, infection control, wound healing, and rapid diagnostics.[10,11] In veterinary and agricultural settings, nanotechnology may reduce excessive dependence on antibiotics through nano-enabled therapeutics, feed additives, vaccines, and biosensors.

Environmental applications, including nanotechnology-based wastewater treatment systems and antimicrobial residue removal technologies, further support AMR mitigation at the ecosystem level.[12] Moreover, the integration of artificial intelligence (AI), smart biosensing systems, and nanoengineering has accelerated the development of advanced precision antimicrobial platforms capable of improving therapeutic efficacy while minimizing toxicity and unnecessary antibiotic exposure.[13]

Therefore, nanotechnology is increasingly recognized as a powerful and versatile strategy capable of addressing AMR from a truly One Health-oriented perspective. However, despite substantial progress, important challenges related to biosafety, toxicity, environmental impact, scalability, regulatory approval, and clinical translation remain unresolved.

This review aims to comprehensively examine the growing AMR crisis through the lens of the One Health framework while critically evaluating the potential of nanotechnology-

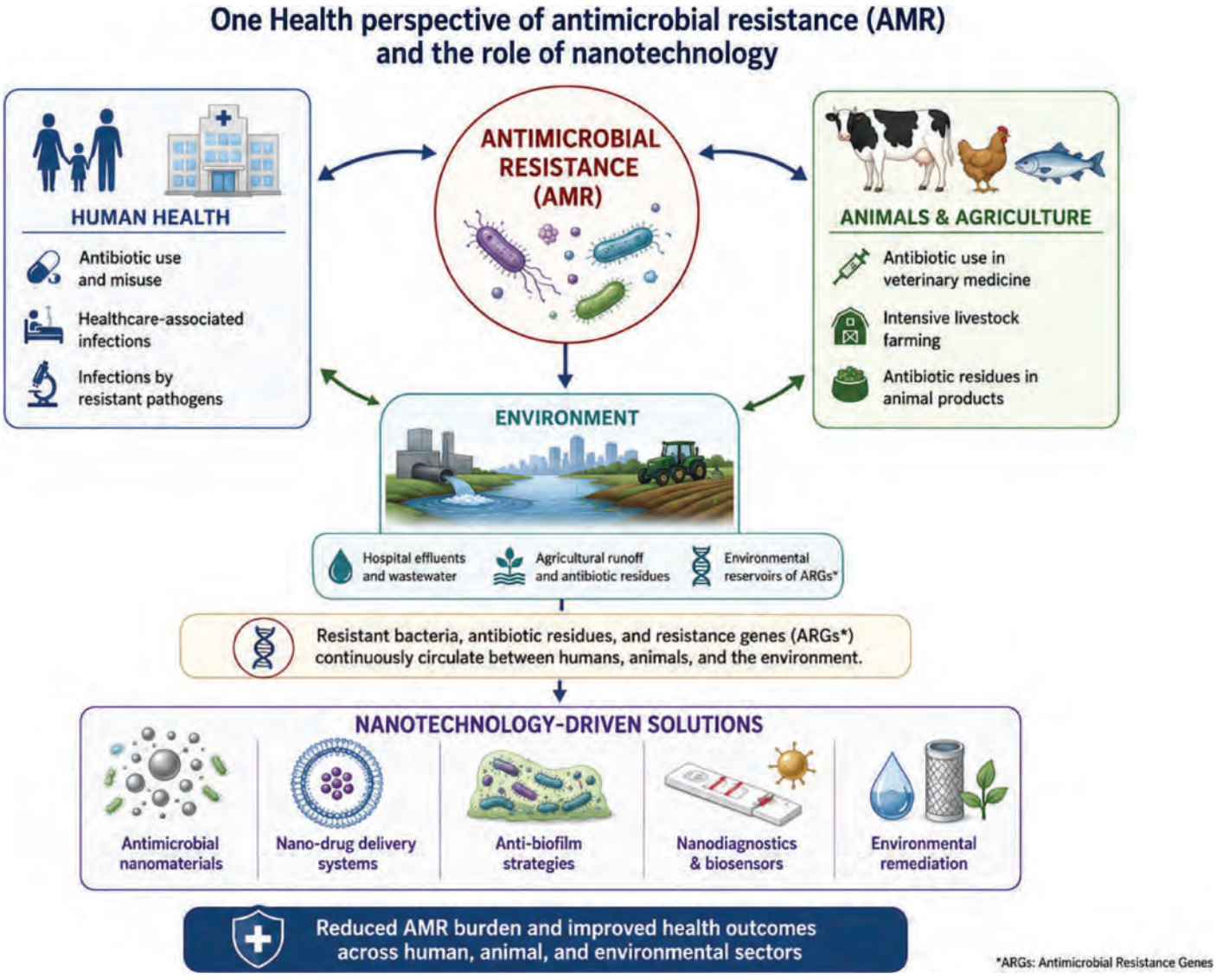

Figure 1. One Health perspective on AMR and the role of nanotechnology. Antimicrobial resistance emerges and spreads through interconnected human, animal, and environmental systems via the circulation of resistant microorganisms, antibiotic residues, and antimicrobial resistance genes (ARGs). A One Health approach integrates these sectors to address AMR through nanotechnology-driven solutions, including antimicrobial nanomaterials, nanodrug delivery systems, antibiofilm strategies, nanodiagnostics and biosensors, and environmental remediation technologies. Together, these approaches contribute to improved antimicrobial stewardship, reduced AMR dissemination, and better health outcomes across human, animal, and environmental domains.

driven strategies as cross-domain solutions. It explores the global burden of AMR, the limitations of conventional antimicrobial approaches, current advances in antimicrobial nanomaterials and nanodrug delivery systems, antibiofilm and nanodiagnostic technologies, applications across human, veterinary, and environmental sectors, integration with emerging technologies such as AI, and future perspectives for sustainable, nano-enabled AMR management.

Figure 1 provides an overview of AMR from a One Health perspective and illustrates how resistant pathogens, antibiotic residues, and antimicrobial resistance genes circulate among humans, animals, and the environment. It also summarizes the major nanotechnology-based strategies discussed throughout this review, highlighting their potential to support integrated AMR prevention, diagnosis, treatment, and environmental remediation within the One Health framework.

THE AMR CRISIS THROUGH THE ONE HEALTH LENS

Human Health Contribution

Human healthcare systems represent one of the primary drivers of the development and dissemination of antimicrobial resistance worldwide. The extensive and frequently inappropriate use of antibiotics in hospitals, outpatient clinics, and community settings has created substantial selective pressure that accelerates the emergence of resistant microorganisms. In many healthcare systems, antibiotics are prescribed unnecessarily for viral infections, such as influenza and the common cold, despite the absence of bacterial pathogens. Empirical broad-spectrum antibiotic therapy, often initiated before a definitive microbiological diagnosis is established, further contributes to resistance selection and microbial adaptation. In addition, self-medication, poor patient adherence, incomplete treatment courses, and unrestricted access to antibiotics in some countries significantly exacerbate the AMR crisis.[14]

The growing prevalence of multidrug-resistant pathogens has become a major challenge in modern clinical practice. Hospital-acquired infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *enterococci* (VRE), and multidrug-resistant *Pseudomonas aeruginosa* are increasingly associated with prolonged hospitalization, increased mortality, and rising healthcare costs.[15] Intensive care units, oncology wards, transplant units, and surgical departments are particularly vulnerable because of the high frequency of invasive procedures, the presence of immunocompromised patients, and extensive antimicrobial exposure.

Another important contributor to AMR in human health is inadequate infection prevention and control. Poor hand hygiene, overcrowded healthcare facilities, insufficient sterilization procedures, and delayed pathogen identification facilitate the transmission of resistant organisms among patients and healthcare workers. International travel and medical tourism further accelerate the global dissemination of resistant pathogens across geographical boundaries.

Biofilm-associated infections constitute another major clinical challenge. Bacterial biofilms protect microorganisms from antibiotics and host immune responses, resulting in persistent infections associated with medical devices, chronic wounds, implanted biomaterials, and indwelling catheters. Within biofilms, microorganisms exhibit altered metabolic states and enhanced resistance to antimicrobial agents, making eradication extremely difficult. Conventional antibiotics often fail to penetrate biofilm matrices effectively, necessitating alternative therapeutic approaches and innovative antimicrobial technologies.[16]

Animal and Agricultural Impact

The animal and agricultural sectors play a major role in the global emergence and dissemination of antimicrobial resistance. Antibiotics are extensively used in livestock production not only for therapeutic purposes but also for disease prevention and growth promotion, particularly in intensive farming systems.[17] Continuous exposure of animals to antimicrobial agents creates strong selective pressure that promotes the survival and proliferation of resistant microorganisms within animal populations. These resistant bacteria may subsequently spread to humans through direct contact with animals, contaminated food products, and environmental pathways.

The large-scale use of medically important antibiotics in the poultry, cattle, swine, and aquaculture industries has raised significant global concern. Resistant pathogens, such as extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and multidrug-resistant *Salmonella* species, have increasingly been detected in food-producing animals.[18] These microorganisms can enter the food chain during slaughter, food processing, transportation, and consumption, thereby facilitating zoonotic transmission and increasing the burden of resistant infections in humans.

Aquaculture systems also contribute substantially to AMR dissemination because of the frequent administration of antibiotics in fish-farming environments. Residual antimicrobial compounds released into aquatic ecosystems may persist for long periods and promote the development of resistance among environmental microorganisms. Similarly, agricultural practices involving manure application and antibiotic-containing fertilizers introduce resistant bacteria and antimicrobial residues into soil ecosystems, creating environmental reservoirs of resistance genes.[19]

Another important concern is the globalization of food trade and animal product distribution, which accelerates the international spread of resistant pathogens across geographical boundaries. Inadequate veterinary oversight, weak antimicrobial stewardship policies, and insufficient surveillance systems in some regions further complicate AMR control efforts in agricultural settings.

Consequently, AMR in animals and agriculture should not be viewed solely as a veterinary issue but rather as a major component of the broader One Health challenge. Effective mitigation strategies require stricter regulations governing antimicrobial use in livestock production, improved farm hygiene and biosecurity measures, enhanced vaccination programs, and the development of sustainable alternatives to antibiotics, including nano-enabled therapeutics, vaccines, and biosensors.

ENVIRONMENTAL RESERVOIRS

Environmental reservoirs play a central role in the persistence, evolution, and dissemination of antimicrobial resistance across ecosystems. Unlike clinical settings, where resistant infections are often detected and monitored, environmental reservoirs allow resistant microorganisms and resistance genes to circulate silently and continuously among humans, animals, water systems, and soil environments. Consequently, the environment functions not only as a passive sink for antimicrobial contaminants but also as an active driver of resistance evolution and transmission.

Wastewater treatment plants, pharmaceutical manufacturing waste, hospital effluents, and agricultural runoff contain antibiotics, resistant bacteria, and resistance determinants that enter natural ecosystems.[20] In many regions, incomplete wastewater treatment processes fail to fully eliminate antimicrobial residues and resistant microorganisms before water is discharged into rivers, lakes, and coastal environments. As a result, aquatic ecosystems become major hotspots for resistance dissemination and microbial adaptation.

Environmental microorganisms may acquire resistance genes through horizontal gene transfer mechanisms, including conjugation, transformation, and transduction. Mobile genetic elements, such as plasmids, transposons, and integrons, facilitate the rapid dissemination of resistance determinants across bacterial populations.[21] Environmental stressors, including heavy metals, disinfectants, and residual antimicrobial compounds, may further increase selective pressure and promote the persistence of resistance genes within microbial communities.

Aquatic systems are particularly vulnerable to AMR dissemination because of contamination from untreated wastewater, industrial discharge, and pharmaceutical pollution. Rivers and surface waters receiving hospital and municipal wastewater often contain high concentrations of resistant bacteria and antimicrobial residues, allowing resistant organisms to spread across large geographical areas. Similarly, groundwater contamination may contribute to the indirect transmission of resistant microorganisms through drinking water systems.

Soil ecosystems also serve as important reservoirs of antimicrobial resistance genes, particularly in areas exposed to intensive agricultural activities, livestock manure application, and antibiotic-containing fertilizers. Resistant bacteria in soil may interact with environmental microorganisms, creating highly dynamic microbial networks that facilitate gene exchange and the long-term persistence of resistance.

Another major concern is the environmental accumulation of antimicrobial compounds and nanoparticles, which may alter natural microbial diversity and ecological balance. The continuous release of antimicrobial agents into ecosystems creates chronic selective pressure that favors resistant microorganisms while suppressing susceptible microbial populations.

Given the complex environmental dimension of AMR, effective mitigation strategies require improved wastewater treatment technologies, stricter pharmaceutical waste management, environmental surveillance systems, and sustainable antimicrobial stewardship practices. Nanotechnology-based environmental remediation systems, advanced filtration membranes, photocatalytic nanoparticles, and nano-enabled biosensors may provide promising tools for reducing antimicrobial contamination and limiting the environmental spread of resistance genes.

WHY CONVENTIONAL STRATEGIES ARE FAILING

Despite decades of scientific progress and the discovery of numerous antimicrobial agents, conventional strategies for combating infectious diseases are becoming increasingly insufficient in the face of rapidly evolving antimicrobial resistance. For many years, the development of new antibiotics was considered the primary solution to resistant infections. However, the extraordinary adaptive capacity of microorganisms has allowed resistance mechanisms to emerge shortly after the introduction of nearly every major antibiotic class. Consequently, resistance is now evolving at a rate that significantly exceeds the pace of novel antibiotic discovery, creating a widening therapeutic gap that threatens the effectiveness of modern healthcare systems.

One of the most critical limitations of conventional antimicrobial approaches is the dramatic slowdown in antibiotic discovery and development. During the golden era of antibiotic discovery in the mid-twentieth century, numerous classes of antibiotics were identified from natural and synthetic sources. In recent decades, however, the development pipeline has declined substantially because of scientific, economic, and regulatory challenges.[4] Pharmaceutical companies have reduced investment in antibiotic research because antimicrobial drugs generally provide lower financial returns than therapies for chronic diseases. Moreover, the rapid emergence of resistance after commercialization often shortens the clinical lifespan of newly introduced antibiotics, further discouraging investment.

At the microbial level, pathogens continuously evolve sophisticated resistance mechanisms that compromise the efficacy of existing drugs. Many antibiotics target specific

bacterial structures or metabolic pathways, allowing microorganisms to adapt through spontaneous mutations or the horizontal acquisition of resistance genes.[22] Common resistance mechanisms include enzymatic degradation of antibiotics, modification of drug targets, reduced membrane permeability, overexpression of efflux pumps, and alterations in metabolic pathways. These adaptive responses enable microorganisms to survive antimicrobial exposure and proliferate even in highly selective environments.

Another major challenge is biofilm-associated resistance. Biofilms are highly organized microbial communities enclosed within extracellular polymeric matrices that provide strong protection against antibiotics and host immune defenses.[23] Within biofilms, microorganisms exhibit altered metabolic activity and enhanced resistance to antimicrobial agents, making eradication extremely difficult. Conventional antibiotics often fail to penetrate biofilm structures effectively, resulting in persistent and recurrent infections associated with medical devices, implants, chronic wounds, and indwelling catheters.

Conventional antibiotics also have several pharmacological and therapeutic limitations, including poor bioavailability, systemic toxicity, nonspecific tissue distribution, and rapid degradation in biological environments.[24] Broad-spectrum antimicrobial agents may disrupt the normal microbiota, increasing the risk of secondary infections and further promoting the selection of resistance. Additionally, excessive antibiotic exposure exerts strong selective pressure on microbial populations, accelerating the emergence and dissemination of resistant strains.

Another important weakness of current AMR management strategies is the lack of coordinated global implementation of One Health principles. Human healthcare systems, veterinary sectors, agricultural industries, food production systems, and environmental agencies frequently operate independently, with insufficient communication, integrated surveillance, and data sharing.[25] Consequently, resistant pathogens and resistance genes continue to circulate across interconnected ecosystems despite local containment efforts.

Diagnostic limitations further complicate AMR control. Conventional microbiological diagnostic methods are often time-consuming and may require several days to identify pathogens and determine antimicrobial susceptibility profiles. Delayed diagnosis frequently results in the empirical use of broad-spectrum antibiotics, which contributes substantially to inappropriate antimicrobial exposure and the development of resistance.

In addition, globalization, international travel, urbanization, climate change, and increasing population density facilitate the rapid worldwide dissemination of resistant microorganisms. Resistant pathogens can spread across continents through human migration, food trade, animal transportation, and environmental contamination, making AMR a truly transnational challenge that cannot be effectively controlled through isolated local interventions.

Collectively, these limitations highlight the urgent need for innovative, multidisciplinary approaches capable of overcoming the shortcomings of conventional antimicrobial strategies. The growing failure of traditional antibiotics has therefore accelerated interest in alternative technologies, such as nanotechnology, precision medicine, artificial intelligence-assisted therapeutics, and advanced biosurveillance systems, which may provide more sustainable solutions for combating AMR within the One Health framework.

NANOTECHNOLOGY AS A ONE HEALTH SOLUTION TO AMR

Antimicrobial Nanomaterials

Antimicrobial nanomaterials represent one of the most extensively investigated areas of nanotechnology-based AMR management because of their remarkable ability to inhibit a broad range of pathogenic microorganisms through diverse and often simultaneous mechanisms of action. Unlike conventional antibiotics, which usually target a single bacterial pathway or cellular structure, nanomaterials can interact with microbial cells through multiple physicochemical processes, thereby reducing the likelihood of resistance development and improving antimicrobial efficacy against multidrug-resistant pathogens.

The antimicrobial activity of nanomaterials is strongly influenced by their size, shape, surface charge, chemical composition, and surface functionalization. At the nanoscale, particles possess exceptionally high surface-area-to-volume ratios that enhance their interactions with bacterial membranes and intracellular structures.[26] These unique properties enable nanoparticles to penetrate microbial cell walls more effectively and disrupt critical cellular functions.

A wide variety of nanomaterials have demonstrated promising antimicrobial activity, including metallic nanoparticles, metal oxide nanoparticles, carbon-based nanomaterials, polymeric nanocomposites, and hybrid nanoformulations. Among these, metallic and metal oxide nanoparticles, such as silver nanoparticles (AgNPs), zinc oxide nanoparticles (ZnO NPs), copper oxide nanoparticles (CuO NPs), titanium dioxide nanoparticles (TiO_2 NPs), and gold nanoparticles (AuNPs), have attracted substantial attention because of their potent broad-spectrum antimicrobial properties.[27,28]

Nanomaterials possess unique physicochemical properties that enable potent antimicrobial activity against resistant

pathogens. Metallic and metal oxide nanoparticles, including AgNPs, ZnO NPs, CuO NPs, TiO_2 NPs, and AuNPs, have demonstrated broad-spectrum antimicrobial effects.[27]

Silver nanoparticles are among the most extensively investigated antimicrobial nanomaterials because of their potent activity against Gram-positive and Gram-negative bacteria, fungi, and viruses. AgNPs exert antimicrobial effects through multiple mechanisms, including reactive oxygen species (ROS) generation, membrane disruption, protein denaturation, and DNA damage.[29]

Zinc oxide nanoparticles exhibit photocatalytic antimicrobial activity and can induce oxidative stress in bacterial cells. Similarly, titanium dioxide nanoparticles generate ROS upon exposure to ultraviolet light, contributing to microbial inactivation.[28] Gold nanoparticles have gained attention because of their excellent biocompatibility and potential applications in photothermal therapy and biosensing.[27]

The multitarget antimicrobial mechanisms of nanoparticles reduce the likelihood of resistance development compared with conventional antibiotics. In addition, nanoparticle surface modification enables enhanced selectivity and synergistic effects when nanoparticles are combined with antibiotics.

The antimicrobial activity of nanomaterials relies on multiple complementary mechanisms rather than a single molecular target. By simultaneously disrupting bacterial membranes, inducing oxidative stress, interfering with intracellular biomolecules, inhibiting biofilm formation, and enhancing targeted drug delivery, nanotechnology offers a multifaceted strategy for combating multidrug-resistant pathogens. An overview of these principal antimicrobial mechanisms is presented in Figure 2.

Nanodrug Delivery Systems

Nanodrug delivery systems represent one of the most important nanotechnology-based strategies for improving antimicrobial therapy against resistant pathogens. Conventional antibiotics often have poor solubility, limited bioavailability, rapid degradation, nonspecific tissue distribution, and systemic toxicity, all of which reduce therapeutic efficacy and contribute to resistance development. Nano-enabled delivery systems overcome many of these limitations by enhancing drug stability, improving targeted delivery, prolonging circulation time, and enabling controlled or stimuli-responsive drug release directly at infection sites. In addition, nanocarriers can improve intracellular penetration and facilitate antimicrobial accumulation within biofilms and infected tissues, thereby increasing treatment effectiveness while minimizing damage to healthy cells and reducing unnecessary antibiotic exposure.

Nanodrug delivery systems improve antimicrobial therapy by enhancing drug stability, solubility, targeted delivery, and controlled release. Liposomes, polymeric nanoparticles, dendrimers, solid lipid nanoparticles, and nanoemulsions have been widely investigated for antimicrobial applications.[24,30]

Liposomes are phospholipid vesicles capable of encapsulating both hydrophilic and hydrophobic antimicrobial agents. Liposomal formulations improve drug pharmacokinetics, reduce systemic toxicity, and enhance intracellular delivery. Polymeric nanoparticles composed of biodegradable polymers, such as poly(lactic-co-glycolic acid) (PLGA), enable sustained drug release and targeted delivery to infected tissues. Functionalized nanoparticles can specifically target bacterial cells or biofilm environments through ligand-mediated interactions.

Nanodelivery systems may also reduce the required antibiotic dosage and minimize the selective pressure that drives resistance evolution. Combination nanotherapies involving antibiotics and metallic nanoparticles have demonstrated synergistic antimicrobial effects against resistant pathogens.[30]

Nanodiagnostics for AMR Detection

Rapid and accurate detection of resistant pathogens is essential for effective antimicrobial stewardship, early therapeutic intervention, and infection control. Conventional microbiological diagnostic methods often require several days to identify pathogens and determine antimicrobial susceptibility profiles, resulting in delayed treatment decisions and increased empirical use of broad-spectrum antibiotics. Consequently, there is a growing need for rapid, sensitive, cost-effective, and portable diagnostic technologies capable of detecting resistant microorganisms in clinical, veterinary, agricultural, and environmental settings.

Nanotechnology-based diagnostic systems have emerged as highly promising tools for improving AMR detection because of their exceptional sensitivity, rapid response times, and potential for miniaturization. Nanodiagnostic platforms use unique nanoscale properties to detect pathogens, resistance genes, toxins, and antimicrobial susceptibility patterns with high precision. These technologies may significantly reduce diagnostic turnaround times and support more targeted antimicrobial therapy, thereby minimizing unnecessary antibiotic exposure and slowing the development of resistance.[31]

Rapid and accurate pathogen identification is essential for appropriate antimicrobial therapy and antibiotic stewardship. Conventional microbiological methods are often time-consuming and may delay treatment decisions.

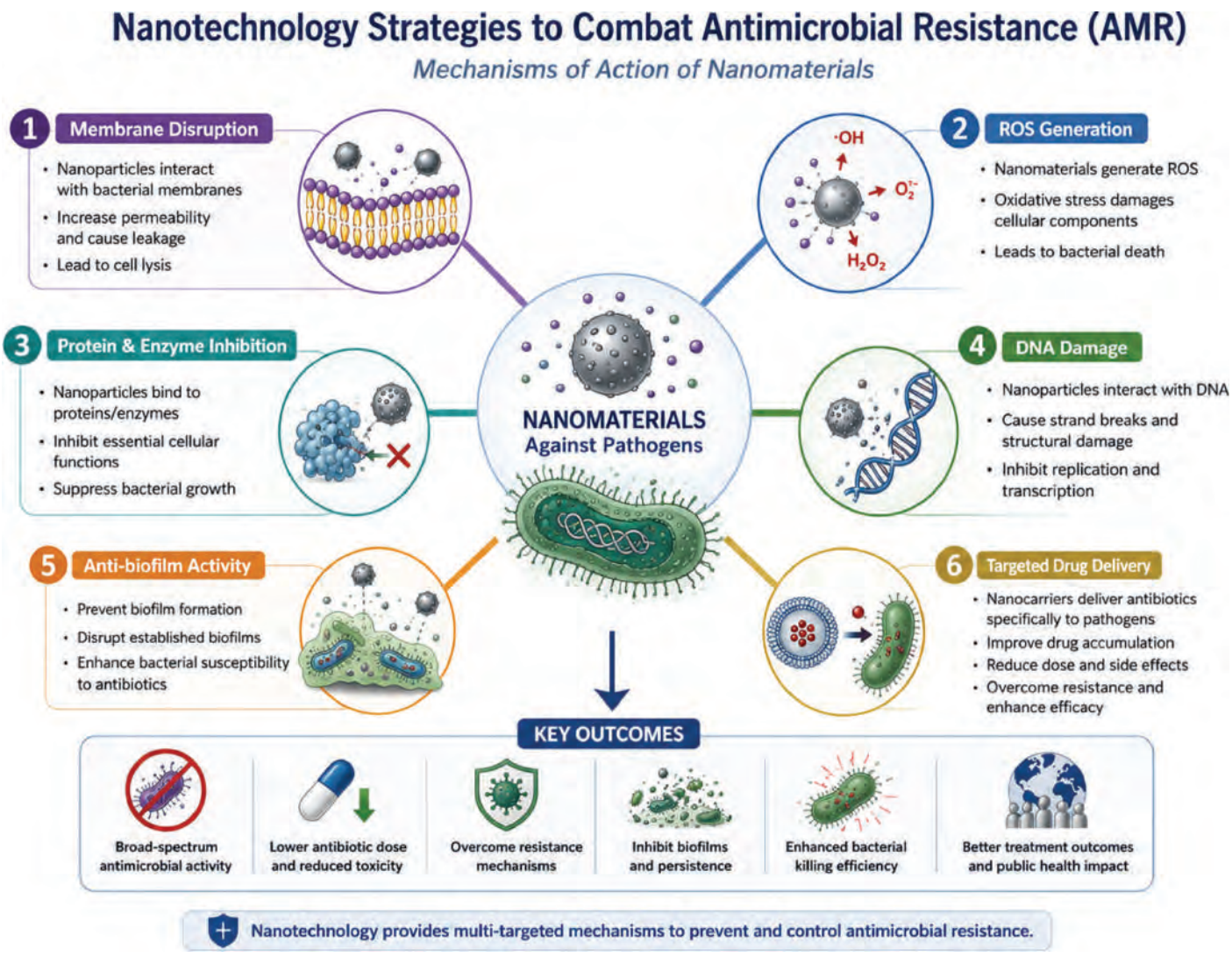

Figure 2. Major antimicrobial mechanisms of nanotechnology-based strategies against antimicrobial-resistant microorganisms. Nanomaterials exert antimicrobial activity through multiple complementary mechanisms, including bacterial membrane disruption, reactive oxygen species (ROS) generation, inhibition of essential proteins and enzymes, nucleic acid damage, biofilm prevention and eradication, and the targeted delivery of antimicrobial agents. Because these mechanisms act simultaneously, nanotechnology can improve bacterial killing efficiency, enhance antibiotic efficacy, reduce the required therapeutic dose, limit toxicity, and decrease the likelihood of resistance development compared with conventional single-target antibiotics.

Nanotechnology-based biosensors and diagnostic systems enable the rapid, sensitive, and portable detection of pathogens and resistance genes.[32] Gold nanoparticles, quantum dots, magnetic nanoparticles, and graphene-based sensors have been used for nucleic acid detection, immunosensing, and surface-enhanced Raman spectroscopy (SERS)-based diagnostics.[11,33–36] These nano-enabled platforms offer several advantages over conventional diagnostic approaches, including high analytical sensitivity, low detection limits, rapid signal amplification, and the ability to detect pathogens directly in complex biological samples. In addition, miniaturized nanobiosensors may support point-of-care testing in hospitals, veterinary clinics, farms, and environmental monitoring systems, thereby improving early detection and facilitating more accurate antimicrobial stewardship strategies.

Point-of-care nanodiagnostic platforms can facilitate the early detection of resistant infections in clinical, veterinary, and environmental settings. Early diagnosis reduces inappropriate antibiotic use and supports precision antimicrobial therapy.

SERS-based nanoplatforms have demonstrated remarkable sensitivity in detecting bacterial fingerprints, resistance biomarkers, and antimicrobial susceptibility patterns.[37–39] These systems use plasmonic nanostructures capable of significantly amplifying Raman scattering signals, thereby enabling ultrasensitive detection or imaging of microbial components even at very low concentrations.[40] SERS-based diagnostics may facilitate the rapid identification of resistant pathogens directly from clinical and environmental samples without requiring lengthy culture procedures.[41] In addition, multiplexed nanoplatforms can simultaneously detect multiple pathogens and resistance genes, improving diagnostic efficiency and supporting more comprehensive surveillance strategies. Integration with artificial intelligence algorithms further enhances diagnostic accuracy, automated data interpretation, and predictive capabilities by enabling the rapid analysis of complex spectral datasets and improving the early detection of emerging resistance patterns.

APPLICATIONS OF NANOTECHNOLOGY ACROSS ONE HEALTH DOMAINS

Human Medicine

Nanotechnology has become an increasingly important tool in human medicine for preventing, diagnosing, and treating infections caused by antimicrobial-resistant pathogens. Unlike conventional antimicrobial therapies, nano-enabled approaches improve therapeutic efficacy by enhancing drug stability, increasing bioavailability, enabling targeted delivery, and reducing systemic toxicity. In addition, many nanomaterials possess intrinsic antimicrobial properties, allowing them to directly inhibit bacterial growth while simultaneously serving as carriers for antibiotics or other therapeutic agents. These multifunctional characteristics make nanotechnology particularly attractive for combating multidrug-resistant (MDR) microorganisms and reducing the excessive use of broad-spectrum antibiotics.[27,30]

One of the most successful clinical applications of nanotechnology is the development of advanced wound dressings and implant coatings containing antimicrobial nanoparticles. Silver nanoparticle-based dressings have demonstrated broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria while promoting wound healing and reducing biofilm formation in chronic wounds.[42] Similarly, nanoengineered coatings applied to orthopedic implants, catheters, prosthetic devices, and cardiovascular implants help prevent bacterial adhesion and significantly reduce the incidence of healthcare-associated infections.[43] Liposomal and polymeric nanoparticle formulations further enhance the intracellular delivery of antimicrobial agents, making them particularly useful for treating persistent infections caused by intracellular pathogens and biofilm-forming bacteria.[44,45]

Recent advances have also expanded the role of nanotechnology in precision medicine. Stimuli-responsive nanocarriers capable of releasing antibiotics in response to pH changes, bacterial enzymes, or inflammatory signals enable localized drug delivery at infection sites while minimizing systemic exposure and adverse effects. In parallel, the integration of nanotechnology with biosensors, molecular diagnostics, and artificial intelligence is facilitating rapid pathogen identification and personalized antimicrobial therapy, thereby supporting antimicrobial stewardship and improving clinical decision-making.[46] These developments highlight the growing potential of nanotechnology to transform the management of resistant infections in modern healthcare systems while aligning with the broader objectives of the One Health approach.

Veterinary Medicine and Agriculture

Nanotechnology offers significant opportunities to reduce antimicrobial use in veterinary medicine and agriculture while maintaining animal health and productivity. Nano-enabled antimicrobial agents, vaccine adjuvants, drug delivery systems, and feed additives have emerged as promising alternatives to the routine use of antibiotics in livestock production.[47] By improving the bioavailability and controlled release of veterinary drugs, nanoformulations can enhance therapeutic efficacy, reduce treatment frequency, and minimize the overall amount of antibiotics administered to food-producing animals.

Nanoparticle-based vaccine delivery systems have also demonstrated considerable potential by improving antigen stability, enhancing immune responses, and providing prolonged protection against infectious diseases.[47] These advances may decrease the incidence of bacterial infections and reduce reliance on prophylactic antibiotic use in intensive farming systems. In addition, nanotechnology has been explored for the development of antimicrobial coatings for animal housing equipment and water systems, which may help reduce pathogen transmission and improve farm biosecurity.

In agriculture, nanotechnology contributes to sustainable food production through the development of nanopesticides, nanofertilizers, and controlled-release agrochemicals that improve resource utilization while reducing environmental contamination.[48] Nano-enabled biosensors can rapidly detect pathogens, toxins, and contaminants on livestock farms and in food production facilities, allowing early intervention before disease outbreaks become widespread. Furthermore, portable nanosensors facilitate real-time monitoring of animal

health and food safety, strengthening surveillance programs and supporting the One Health approach to antimicrobial resistance management.[31]

Overall, the integration of nanotechnology into veterinary medicine and agricultural practices has the potential to reduce excessive dependence on antibiotics, improve disease prevention, enhance food safety, and limit the dissemination of resistant microorganisms from animals to humans and the environment. Continued research, regulatory oversight, and field validation will be essential to ensure the safe, effective, and sustainable implementation of these nano-enabled technologies.

Environmental Applications

Environmental remediation represents a critical component of the One Health approach to combating antimicrobial resistance because environmental compartments serve as important reservoirs of antibiotic residues, resistant bacteria, and antimicrobial resistance genes. Nanotechnology-based wastewater treatment systems have shown considerable potential for removing these contaminants from hospital effluents, pharmaceutical wastewater, municipal sewage, and agricultural runoff before they are released into natural ecosystems.[49] By reducing environmental contamination, these technologies help interrupt the transmission cycle of resistant microorganisms among the environment, animals, and humans.

Photocatalytic nanoparticles, such as TiO_2 and ZnO, can generate reactive oxygen species under light irradiation, leading to the degradation of antibiotic residues and effective microbial inactivation. Magnetic nanoparticles facilitate the adsorption and removal of antibiotics, heavy metals, and other environmental pollutants and can be easily recovered using external magnetic fields, making them attractive for large-scale water treatment applications.[50] In addition, nano-enabled filtration membranes, nanocomposite adsorbents, and biosorbent materials have demonstrated promising performance in wastewater purification by improving filtration efficiency, reducing membrane fouling, and enhancing the removal of resistant microorganisms and resistance genes.[51]

Recent advances have also focused on integrating nanotechnology with environmental monitoring systems. Nanotechnology-based biosensors enable the rapid and sensitive detection of antibiotic residues, pathogenic microorganisms, and antimicrobial resistance genes in water, soil, and food production environments. These technologies support continuous environmental surveillance, allowing the early identification of contamination events and facilitating timely interventions. As environmental monitoring becomes increasingly important within the One Health framework, nano-enabled sensing platforms may play a key role in preventing the environmental dissemination of AMR and supporting sustainable ecosystem management.[31]

Integration with Emerging Technologies

The convergence of nanotechnology with emerging digital and computational technologies is transforming AMR management by enabling more precise diagnosis, targeted therapy, predictive surveillance, and personalized treatment strategies. Recent advances in artificial intelligence (AI), machine learning (ML), the Internet of Things (IoT), big data analytics, robotics, and advanced biosensing technologies have significantly expanded the capabilities of nano-enabled antimicrobial platforms. Rather than functioning independently, these innovations increasingly complement one another to create integrated systems capable of addressing AMR across the interconnected human, animal, and environmental sectors within the One Health framework.[52,46]

Artificial intelligence has become a powerful tool for accelerating the design and optimization of antimicrobial nanomaterials. Traditional nanoparticle development often requires extensive laboratory experimentation involving numerous synthesis parameters, making the process time-consuming and costly. AI-driven computational models can analyze large experimental datasets to predict how nanoparticle size, morphology, surface charge, composition, and functionalization influence antimicrobial activity, toxicity, cellular uptake, and biodistribution.[46,53] Machine learning algorithms further assist in identifying optimal nanoparticle formulations by recognizing complex relationships between physicochemical characteristics and biological performance that may not be evident through conventional statistical analyses. Consequently, AI-supported nanoparticle engineering reduces the experimental workload while facilitating the rational development of safer and more effective nanotherapeutics.

Machine learning has also become increasingly important for analyzing complex biological and spectroscopic datasets generated during pathogen identification and antimicrobial susceptibility testing. High-dimensional datasets obtained from genomic sequencing, proteomics, metabolomics, and spectroscopic techniques such as SERS can be rapidly processed using supervised and unsupervised learning algorithms. These computational approaches improve pathogen classification, identify antimicrobial resistance biomarkers, predict resistance phenotypes, and support clinical decision-making with high diagnostic accuracy. As more clinical datasets become available, AI-based predictive models are expected to play an increasingly important

role in precision antimicrobial therapy by recommending individualized treatment strategies based on pathogen characteristics and patient-specific factors.[7]

Smart nanodrug delivery systems represent another major advancement resulting from the integration of nanotechnology with materials science and biomedical engineering. Stimuli-responsive nanoparticles can selectively release antimicrobial agents in response to environmental triggers, such as acidic pH, bacterial enzymes, temperature changes, oxidative stress, inflammatory mediators, or bacterial toxins present at infection sites. This controlled drug release minimizes systemic toxicity while maximizing local antimicrobial concentrations, thereby improving therapeutic efficacy and reducing unnecessary antibiotic exposure. Furthermore, multifunctional nanoplatforms capable of simultaneously delivering antibiotics, anti-inflammatory agents, and biofilm-disrupting compounds provide promising opportunities for treating chronic and multidrug-resistant infections more effectively than conventional therapies.[54]

The integration of nanotechnology with advanced biosensing technologies has also revolutionized infectious disease diagnostics. Nano-enabled biosensors incorporating gold nanoparticles, magnetic nanoparticles, quantum dots, graphene, and other nanomaterials provide exceptional analytical sensitivity and enable the rapid detection of pathogens, antimicrobial resistance genes, and microbial toxins.[32] Compared with conventional microbiological culture methods, these platforms substantially reduce diagnostic turnaround times while enabling the multiplex detection of several pathogens in a single assay. Such capabilities are particularly valuable in clinical settings, where early diagnosis supports timely antimicrobial intervention and reduces the inappropriate use of broad-spectrum antibiotics.

Recent advances in SERS further demonstrate the benefits of combining nanotechnology with computational approaches. SERS uses plasmonic nanostructures to amplify Raman scattering signals by several orders of magnitude, enabling ultrasensitive detection of bacterial biochemical fingerprints, resistance biomarkers, and antimicrobial susceptibility profiles.[55] When combined with machine learning algorithms, SERS datasets can be automatically analyzed to classify bacterial species, distinguish resistant isolates from susceptible isolates, and identify subtle molecular changes associated with the evolution of resistance.[7,56] This integration has shown considerable promise for the development of rapid, culture-independent diagnostic systems capable of supporting precision medicine and antimicrobial stewardship.

Beyond clinical applications, IoT-enabled nanosensors are emerging as valuable tools for the continuous monitoring of antimicrobial resistance across multiple One Health environments. Networks of portable nano-enabled sensors can continuously monitor hospitals, livestock farms, food production facilities, wastewater treatment plants, and natural water systems for the presence of antibiotic residues, resistant microorganisms, and resistance genes. The collected data can be transmitted in real time to centralized surveillance systems, where AI algorithms analyze trends, identify emerging outbreaks, and support evidence-based public health interventions. Such integrated surveillance networks may substantially strengthen global AMR preparedness while improving communication among healthcare providers, veterinarians, environmental agencies, and public health authorities.[57]

Despite these promising advances, several important challenges remain before these integrated technologies can be widely implemented. Large, standardized, high-quality datasets are required to train reliable AI models, while data privacy, cybersecurity, algorithmic transparency, and interoperability among digital healthcare systems remain significant concerns. Moreover, regulatory frameworks governing AI-assisted diagnostics and nano-enabled medical devices continue to evolve, requiring international harmonization to facilitate clinical translation. The long-term safety of smart nanomaterials, ethical considerations surrounding AI-assisted decision-making, and equitable access to advanced technologies must also be carefully addressed.

Overall, the integration of nanotechnology with artificial intelligence, machine learning, advanced biosensing technologies, and the IoT represents one of the most promising directions for future AMR management. By combining rapid diagnostics, intelligent therapeutic design, precision drug delivery, and real-time surveillance within a unified One Health framework, these emerging technologies have the potential to fundamentally transform how resistant infections are detected, monitored, and treated worldwide.

The future management of antimicrobial resistance will increasingly rely on the integration of nanotechnology with artificial intelligence, machine learning, biosensors, and digital surveillance systems within the One Health framework. By combining rapid pathogen detection, intelligent data analysis, targeted nanotherapeutics, and coordinated decision-making across human, animal, and environmental sectors, these technologies provide a comprehensive strategy for precision AMR management, as illustrated in Figure 3.

Figure 3. Conceptual framework of AI-enabled nanotechnology for precision AMR management within the One Health framework. Data generated across interconnected human, animal, food, and environmental sectors are collected through nano-enabled biosensors and integrated into artificial intelligence (AI) and machine learning (ML) platforms for rapid analysis, prediction, and decision support. The resulting information guides precision nanotherapeutics, antibiofilm strategies, combination therapies, and environmental remediation while strengthening antimicrobial stewardship, surveillance, and coordinated One Health interventions. This integrated ecosystem supports the earlier detection of resistant pathogens, optimization of treatment strategies, reduction of AMR transmission, and improvement of public health outcomes.

CHALLENGES AND LIMITATIONS

Despite the remarkable progress achieved in nanotechnology-based antimicrobial research, several important scientific, technical, regulatory, economic, and environmental challenges continue to limit the widespread implementation of nano-enabled strategies for combating AMR. Although numerous nanomaterials have demonstrated promising antimicrobial efficacy under laboratory conditions, relatively few have successfully progressed to clinical practice or large-scale environmental applications. Bridging this gap requires a comprehensive understanding of nanoparticle safety, manufacturing, regulatory approval, and long-term performance across diverse One Health settings.

One of the primary concerns is the potential toxicity of nanomaterials. Although nanoparticles can selectively target microbial cells, they may also interact with mammalian cells and potentially induce oxidative stress, inflammation, DNA damage, mitochondrial dysfunction, and apoptosis, depending on their size, composition, surface chemistry, concentration, and duration of exposure.[58] Certain metallic nanoparticles may accumulate in organs such as the liver, kidneys, lungs, or spleen following repeated administration, raising concerns about long-term biosafety and chronic toxicity. Furthermore, interactions between nanoparticles and biological systems remain highly complex, making comprehensive toxicological evaluation essential before clinical implementation.

Another significant challenge is the environmental fate and ecotoxicological impact of engineered nanomaterials. The increasing production and use of nanoparticles inevitably lead to their release into wastewater systems, agricultural soils, and aquatic environments. Once released, nanoparticles may persist, aggregate, dissolve, or undergo physicochemical transformations that influence their biological activity and environmental behavior. Their accumulation may alter microbial community composition, affect beneficial microorganisms involved in nutrient cycling, and disrupt ecological balance. Consequently, life-cycle assessment and environmental risk evaluation should become integral components of future nanotechnology development.[59]

The large-scale manufacturing and reproducibility of nanomaterials also remain major obstacles to commercialization. Laboratory-scale synthesis methods often produce nanoparticles with highly controlled physicochemical properties; however, maintaining the same particle size distribution, morphology, surface characteristics, and stability during industrial-scale production remains technically challenging. Batch-to-batch variability can significantly influence antimicrobial activity, pharmacokinetics, and safety profiles, emphasizing the need for standardized manufacturing protocols and rigorous quality-control procedures.

Economic considerations further affect the clinical translation of nanotechnology. The synthesis of high-quality functionalized nanoparticles frequently requires specialized equipment, expensive raw materials, and complex fabrication procedures that increase production costs. In low- and middle-income countries, where the burden of antimicrobial resistance is often greatest, the high cost of nano-enabled therapeutics and diagnostic systems may limit accessibility. Therefore, developing cost-effective, scalable, and sustainable manufacturing strategies remains an important research priority.

Regulatory approval represents another major barrier to widespread implementation. Existing regulatory frameworks were primarily developed for conventional pharmaceuticals and medical devices and are often insufficient to address the unique characteristics of nanomaterials. Regulatory agencies continue to refine guidelines regarding nanoparticle characterization, toxicological assessment, environmental safety, pharmacokinetics, manufacturing quality, and postmarketing surveillance. The lack of internationally harmonized regulatory standards creates uncertainty for manufacturers and slows the translation of promising nanotechnologies into clinical and commercial applications.

Another emerging concern is the possibility that microorganisms may eventually develop adaptive responses to certain nanomaterials. Although nanoparticles generally attack microbial cells through multiple mechanisms, thereby reducing the likelihood of resistance compared with conventional antibiotics, prolonged or inappropriate exposure could still promote microbial adaptation. Changes in membrane composition, activation of oxidative stress defense systems, enhanced biofilm formation, increased efflux pump activity, and altered metabolic pathways have all been proposed as potential adaptive responses to nanoparticle exposure. Continuous surveillance and the rational application of nanomaterials will therefore be necessary to minimize the risk of future nanoresistance.

The clinical evaluation of nano-enabled antimicrobial therapies also remains limited. Most published studies have been conducted under in vitro conditions or in animal models, whereas relatively few large-scale clinical trials have assessed long-term efficacy and safety in human populations. Differences between laboratory conditions and real-world clinical environments may significantly influence therapeutic performance, highlighting the need for multicenter clinical studies involving diverse patient populations before routine medical implementation.

From a One Health perspective, the successful implementation of nanotechnology also requires stronger interdisciplinary collaboration among clinicians, microbiologists, veterinarians, engineers, environmental scientists, toxicologists, regulatory authorities, and policymakers. Effective communication among these disciplines is essential to ensure that nanotechnology-based interventions are not only scientifically effective but also environmentally sustainable, economically feasible, and socially acceptable. International cooperation will further facilitate harmonized regulations, standardized safety assessments, and coordinated surveillance programs for monitoring the long-term impact of nanotechnology on antimicrobial resistance.

Overall, although nanotechnology offers enormous potential for addressing the global AMR crisis, overcoming these scientific and practical limitations will be essential for its successful translation into routine healthcare, veterinary medicine, agriculture, and environmental management. Continued investment in multidisciplinary research, standardized evaluation methods, robust regulatory frameworks, and long-term biosafety studies will play a crucial role in ensuring that nano-enabled technologies can be implemented safely and effectively within the One Health framework.

FUTURE PERSPECTIVES

Future advances in nanotechnology-based strategies for combating AMR will rely on the integration of materials

science, biotechnology, artificial intelligence (AI), and precision medicine within the One Health framework. Although numerous nano-enabled antimicrobial platforms have demonstrated promising results under laboratory conditions, their successful translation into clinical practice, veterinary medicine, agriculture, and environmental management requires further improvements in biosafety, scalability, regulatory approval, and cost-effectiveness.

One of the primary research priorities is the development of safer, biodegradable, and environmentally sustainable nanomaterials with enhanced antimicrobial selectivity and reduced toxicity.[60] Future nanoparticle design should focus on optimizing physicochemical properties, such as size, morphology, surface chemistry, and biodegradability, to improve therapeutic efficacy while minimizing adverse effects. Multifunctional nanoplatforms capable of combining targeted drug delivery, antimicrobial activity, biofilm disruption, controlled drug release, and diagnostic functions may provide highly effective theranostic systems for managing resistant infections.

Artificial intelligence and machine learning are expected to further accelerate nanoparticle development by predicting antimicrobial performance, toxicity, and biological interactions before laboratory synthesis.[53] AI-assisted analysis of genomic, proteomic, and spectroscopic datasets will also improve pathogen identification, resistance prediction, and personalized antimicrobial therapy. In parallel, stimuli-responsive nanodrug delivery systems that release antimicrobial agents in response to infection-specific signals may enhance treatment precision while reducing systemic toxicity and unnecessary antibiotic exposure.

Future One Health strategies will increasingly depend on integrating nanotechnology with advanced biosensors, IoT technologies, and AI-driven surveillance systems. Nano-enabled sensors capable of continuously monitoring antibiotic residues, resistant microorganisms, and resistance genes across hospitals, farms, wastewater treatment plants, and environmental ecosystems may strengthen global AMR surveillance and facilitate the early detection of outbreaks.

Despite these promising developments, important challenges remain, including regulatory approval, large-scale manufacturing, quality control, long-term biosafety, and environmental risk assessment. Addressing these limitations through multidisciplinary collaboration, international regulatory harmonization, and continued translational research will be essential for transforming nanotechnology into a practical and sustainable solution for combating AMR. Overall, the integration of nanotechnology with emerging digital technologies offers significant potential to improve antimicrobial therapy, surveillance, and infection control, supporting more effective AMR management across interconnected human, animal, and environmental sectors.

CONCLUSION

Antimicrobial resistance has evolved into a complex global challenge that extends beyond healthcare and affects interconnected human, animal, and environmental systems. The growing failure of conventional antibiotics, driven by rapid microbial adaptation, biofilm formation, and the limited development of new antimicrobial agents, highlights the need for innovative strategies that address AMR from a broader One Health perspective. Nanotechnology offers significant advantages by providing multifunctional platforms capable of enhancing antimicrobial activity, improving targeted drug delivery, disrupting biofilms, and enabling the rapid and sensitive detection of pathogens.

Recent advances in antimicrobial nanomaterials, nanodrug delivery systems, antibiofilm technologies, and nanodiagnostics demonstrate the potential of nanotechnology to complement existing antimicrobial therapies while supporting more effective antimicrobial stewardship. Moreover, the integration of nanotechnology with artificial intelligence, machine learning, and advanced biosensing technologies is creating new opportunities for precision medicine, intelligent diagnostics, and real-time surveillance of resistant pathogens across multiple One Health environments.

Despite these promising developments, several challenges remain, including long-term biosafety, environmental impact, manufacturing scalability, regulatory approval, and clinical translation. Addressing these limitations through interdisciplinary collaboration and continued translational research will be essential for the successful implementation of nano-enabled technologies. Overall, nanotechnology represents a versatile and promising platform that can complement conventional antimicrobial strategies and contribute substantially to sustainable AMR control within the One Health framework.

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From Mouth to Mind: Unraveling the Oral Microbiome's Role in Orofacial Pain and Neurodegeneration

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ABSTRACT

The human oral cavity hosts a diverse and dynamic microbiome comprising more than 700 microbial species, which plays a crucial role in maintaining oral and systemic health. Oral dysbiosis, defined as disruption of this microbial balance, has been increasingly implicated not only in periodontal disease but also in the pathogenesis of orofacial pain and neurodegenerative diseases. Key pathogens, such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Fusobacterium nucleatum*, have been proposed to release virulence factors, including lipopolysaccharides and gingipains, which may activate immune pathways and nociceptors, thereby driving peripheral sensitization and neuroinflammation. These organisms have also been suggested to breach mucosal and vascular barriers, enter the systemic circulation, and, in some cases, access the central nervous system. Evidence has linked oral pathogens to Alzheimer's disease, Parkinson's disease, autism spectrum disorder, and multiple sclerosis. Notably, *P. gingivalis* and its gingipains have been identified in postmortem brain tissue from patients with Alzheimer's disease, with proposed roles in neuroinflammation and amyloid plaque formation. Salivary microbial alterations in burning mouth syndrome and temporomandibular disorders further highlight the influence of the oral microbiome on neuropathic pain. Therefore, the oral-gut-brain axis represents a novel and promising area of investigation, offering potential diagnostic biomarkers and targeted microbial therapies for the management of chronic orofacial pain and neurological disorders.

Keywords: Brain-gut axis, microbiota-gut-brain axis, neurodegenerative diseases, neuroinflammation, orofacial pain, periodontal pathogens.

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INTRODUCTION

The relationship between the gut and the brain in the context of mental health disorders has become a subject of growing scientific inquiry. Because the oral cavity is the anatomical entry point of the digestive system, researchers have increasingly focused on the potential role of oral microbial communities in mental health outcomes.[1,2]

The oral and gut microbiomes are among the most structurally diverse and microbially rich ecosystems in the human body.[1] A substantial body of research has focused on understanding the gut-brain-microbiome axis, a continuous, bidirectional communication network linking gut

microbial activity to central nervous system (CNS) function. This network operates through neural, endocrine, and immunological pathways, and disruptions within it have been implicated in a range of CNS-related conditions.[2]

The relationship between the gut and the brain extends further, intersecting meaningfully with oral cavity health. The oral environment contains a wide range of microhabitats, including the tongue, gingiva, buccal mucosa, palatal surfaces, and tooth structures, and is the body's second most densely populated microbial niche.[3] Emerging data indicate that gut microbial states may influence oral health outcomes, including the development of dental caries. Cariogenic biofilm communities drive caries initiation, and gut-derived dysbiosis may impair immune regulation in ways that reduce the host's ability to manage these oral pathogens, particularly in pediatric populations.[4]

Of particular note is the expanding body of evidence linking the oral microbiota, which serves as the microbiome's initial interface with the digestive tract, to neurological health outcomes. Because the oral cavity is readily accessible, its microbial profile may serve as a diagnostic indicator of both local and distant diseases, including conditions affecting the nervous system.[5]

The concept of an oral-gut-brain axis has now emerged in the literature. This framework proposes that microorganisms native to the mouth may translocate to the gastrointestinal environment, seeding ectopic colonies that disrupt resident microbial populations. Beyond direct colonization, metabolic products and structural components of oral bacteria may affect brain function either through neural pathways or by entering the systemic circulation. Just as the gut microbiome requires ecological stability to maintain health, the oral microbial ecosystem also depends on microbial balance. Mapping the interactions between host biology and resident microbiota is therefore essential for understanding disease mechanisms and designing microbiome-based approaches to diagnosis and treatment.

Although the oral cavity is anatomically continuous with the gastrointestinal tract, its microbial community differs substantially from that of the gut. Physical compartmentalization by the stomach and small intestine, combined with chemical defenses, including gastric acid and bile salts, limits the direct passage of microbes between the two sites.[6] Even so, oral bacteria can reach the gut, where their presence may shape both local and systemic physiology. Global metagenomic analyses of paired salivary and fecal samples have shown that certain oral bacterial taxa can establish themselves in the intestinal environment, even under conditions of gut health.

Neuropsychiatric conditions, such as depression, autism spectrum disorder (ASD), and schizophrenia, tend to follow a chronic disease course and are frequently accompanied by comorbid health conditions, complicating treatment and contributing to poor long-term outcomes.[7] Depression and anxiety have emerged as particularly pressing public health challenges, with prevalence estimates suggesting that approximately 15–20% of the global population is affected.[5]

A rapidly growing body of evidence identifies the gut microbiome as a key contributor to brain health, with possible roles in neurodevelopment, neurotransmitter modulation, and behavioral regulation. These microbial influences may be relevant to the pathogenesis and progression of neurodevelopmental, psychiatric, and neurodegenerative conditions.[8]

CLINICAL AND RESEARCH IMPLICATIONS

Oral-Gut Microbial Transmission

Neural Pathways

Recent studies have revealed a compelling link between oral microbes and gut health. Although the oral and intestinal microbiomes are generally distinct in healthy individuals.[9] Nearly one-third of oral bacteria may establish themselves in the gut under specific conditions.[10] This transmission is more pronounced in gastrointestinal disorders, in which microbial balance is already disrupted. In periodontitis, oral bacteria can disturb the gut flora, triggering inflammation and further complications.[11] Notably, *Porphyromonas gingivalis* compromises the intestinal barrier by reducing tight junction proteins, including occludin and TJP1, and increasing pro-inflammatory markers, such as IL-6 and IFN-γ.[12] Another oral bacterium, *Streptococcus salivarius*, has also been shown to weaken duodenal epithelial integrity, as demonstrated in a single observational study of patients with functional dyspepsia. However, a causal relationship has not been definitively established.

Immune Pathways

A growing body of evidence links periodontal disease to inflammatory bowel conditions, including Crohn's disease and ulcerative colitis. Oral bacteria are suggested to be capable of migrating to and establishing themselves in the gut, where they may aggravate or possibly even initiate inflammation. Multiple studies have documented shared microbial signatures, such as increased *Prevotella*, *Veillonella*, and *S. salivarius*, in both the oral cavities and intestines of individuals with Crohn's disease.[6] Many studies and meta-analyses are human-based, which strengthens the level of evidence.

Oral bacteria access the gastrointestinal system through two main routes. The first is via the bloodstream, through which microbes such as *Fusobacterium nucleatum* can travel to distant sites. This bacterium has been linked to colorectal cancer, initially identified by Kostic et al.[13] Subsequent studies have confirmed its association with worse outcomes and reduced treatment response. *F. nucleatum* appears to promote tumor development by altering the immune microenvironment and stimulating cancer cell growth. Research on *F. nucleatum* and colorectal cancer includes both human and animal studies; however, human clinical studies and observational analyses are more numerous in identifying the association and clinical significance. Animal studies, however, are crucial and predominant for establishing causation, clarifying functional mechanisms, and testing treatment efficacy.

Microbial and Metabolic Pathways

The second pathway is gastrointestinal. Disruptions, such as antibiotic use or proton pump inhibitor therapy, create opportunities for oral bacteria to thrive in the gut. In addition, Th17 cells primed in inflamed periodontal tissues may enter the systemic circulation and reactivate upon encountering familiar microbial triggers, thereby perpetuating inflammation. Notably, direct colonization may not be necessary for oral bacteria to exert systemic effects. Oral microbiome imbalances driven by poor oral hygiene, diet, medications, or reduced salivation can cause broader physiological disturbances. One affected pathway involves nitric oxide (NO) signaling, which is critical for gut motility. Periodontitis has been linked to reduced systemic levels of NO and BH4, potentially altering intestinal function through the BH4/NO/Nrf2 pathway and contributing to symptoms such as constipation.[6] Epidemiological and observational studies using large databases, such as NHANES, have shown that individuals with periodontitis have significantly higher odds, approximately 45% higher, of experiencing chronic constipation. In animal studies, scientists have established causation by using knockout mice, which are born without the Nrf2 gene, and comparing them with healthy mice. In humans, primarily strong associations have been demonstrated; however, there are not yet enough large-scale clinical trials to confirm causality. In the present scenario, evidence relies heavily on animal models to establish mechanistic causality, creating both opportunities for deeper molecular understanding and limitations in direct human translation.

Mechanism of the Oral-Gut-Brain Axis

Neural Pathways

The brain, gut, and oral cavity are interconnected through neural pathways, with the central nervous system and enteric nervous system (ENS) in constant communication. The vagus nerve serves as the primary conduit, transmitting sensory information from the gut to the brain and relaying motor commands in return. Embedded within the gastrointestinal lining, the ENS, often referred to as the "second brain," governs digestive function while also influencing mood and behavior through vagal and spinal connections.[11]

Sensory signals from the oral cavity, including pain, temperature, and tactile stimuli, travel through the trigeminal nerve (cranial nerve V) and the trigeminal ganglion to the brainstem before ascending to higher processing centers.[12] Oral microbiome dysbiosis can activate this pathway, triggering local or referred pain. Furthermore, persistent oral infections promote systemic inflammation and are increasingly implicated in neuroinflammatory and neurodegenerative conditions. Given the abundance of animal models and *in vitro* studies, this association has been proposed based on anatomical proximity; however, direct human evidence remains lacking. Human evidence is largely observational and indirect. Neither pathway has been definitively proven in humans.

Immune Pathways

Disruptions in the oral microbiota have downstream effects on gut and brain health. When oral bacteria such as *F. nucleatum* travel to the digestive tract, they can impair enteric glial cells, weaken the intestinal lining, and trigger chronic inflammation, potentially affecting cognitive function. Similarly, *P. gingivalis* can disturb gut microbial balance and alter immune responses beyond the oral environment.

The immune system plays a pivotal role in the oral-gut-brain axis. Beneficial gut microbes produce short-chain fatty acids that support brain function, but a compromised gut barrier, often referred to as "leaky gut," allows toxins and harmful molecules to enter the bloodstream, adversely affecting both physical health and emotional well-being (Fig. 1).

THE ORAL-GUT-BRAIN AXIS: IMPLICATIONS FOR NEUROLOGICAL AND MENTAL HEALTH

Mechanisms by Which Oral Microbiota Affect Brain Function

Neural Pathways

A growing body of evidence suggests that the translocation of oral microbes to the brain plays a significant role in the development of neuroinflammatory conditions. Oral microbes can translocate to the brain through neural pathways, including the trigeminal nerve and olfactory system, both of which directly link the oral cavity to the olfactory bulb.[4] When the oral mucosal barrier is compromised, as in chronic periodontitis, bacteria and their components, such as lipopolysaccharides, enter the bloodstream and activate endothelial cells through inflammatory cytokine receptors, including TNF-α and IL-1β. This

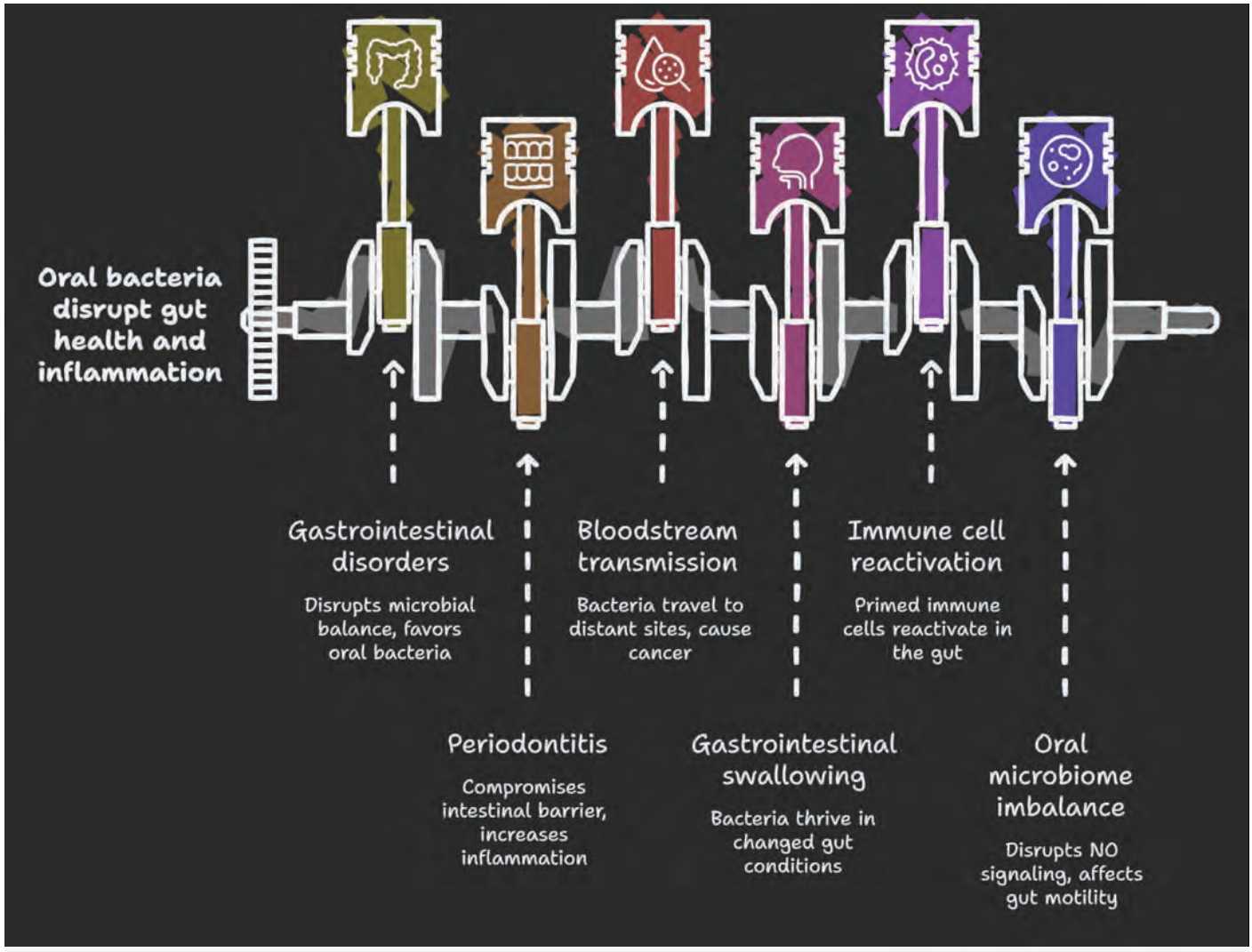

Figure 1. Interactions between the oral microbiome and the gut.

alerts perivascular macrophages, initiates a neuroinflammatory cascade, and activates the hypothalamic-pituitary-adrenal axis, which is closely associated with mood and cognition.

Inflammatory signaling also degrades the blood-brain barrier (BBB), a critical defense against harmful substances, and BBB breakdown has been linked to cognitive disorders and neurodegeneration. *P. gingivalis* exemplifies this threat (Fig. 2). It can breach the BBB, reach brain tissue, and release gingipains, enzymes that activate microglial cells and accelerate dopaminergic neuronal degeneration.[14]

Microbial and Metabolic Pathways

Beyond direct neural invasion, oral microbes influence brain health through the oral-gut-brain axis. Continuously swallowed saliva carries oral bacteria into the gastrointestinal tract, where, particularly after antibiotic use or in immunocompromised individuals, they may alter the composition of the gut microbiota, activate immune responses, and promote chronic low-grade inflammation. This weakens the gut lining, increases intestinal permeability, often referred to as "leaky gut," allows toxins and pro-inflammatory molecules to enter the bloodstream, further disrupts the BBB, impairs nutrient delivery, and ultimately compromises cognition.[3]

Studies have detected elevated levels of oral-origin bacteria in fecal samples, which may be explained by two hypotheses. The "expansion hypothesis" proposes active colonization and multiplication of oral microbes within the gut, whereas the "marker hypothesis" suggests that these microbes merely pass through, with their elevated presence reflecting a reduction in native gut bacteria rather than true colonization. In studies

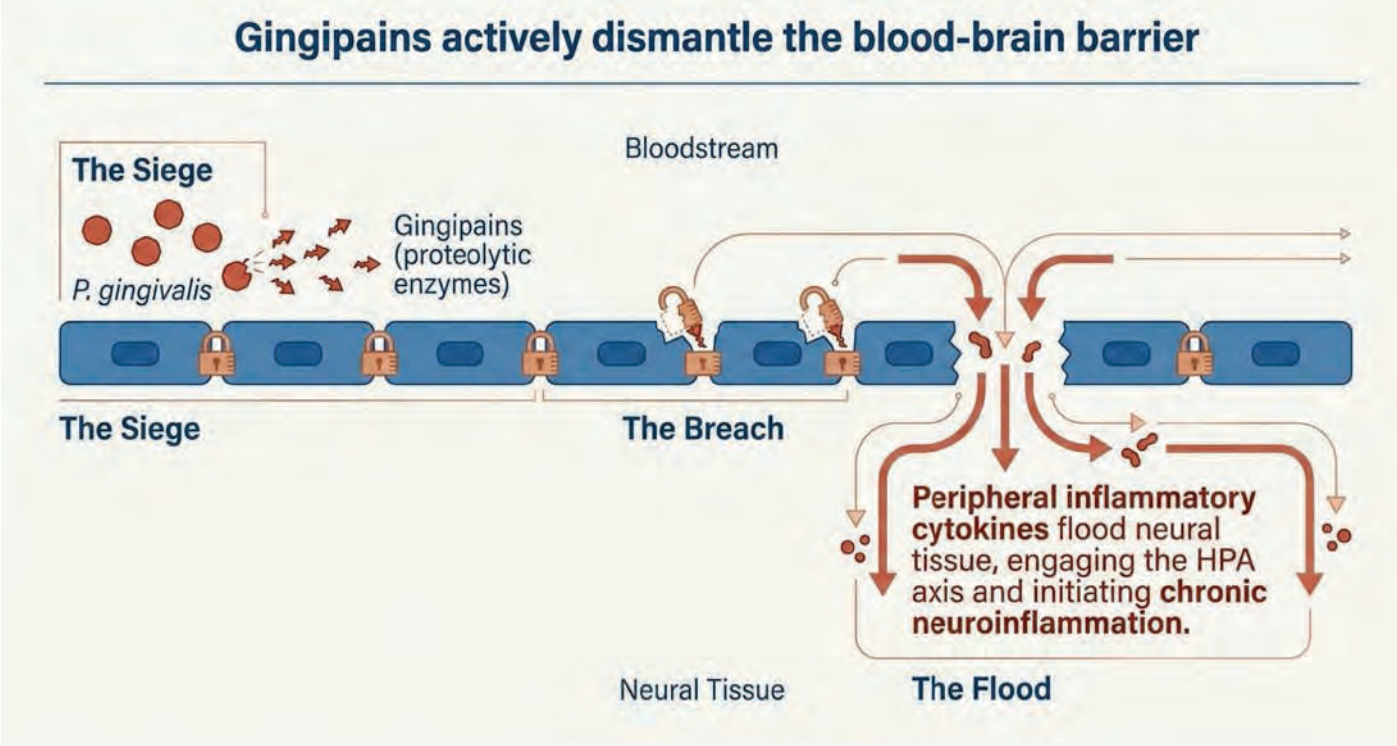

Figure 2. Mechanisms by which gingipains disrupt the blood-brain barrier.

of the oral-gut-brain axis in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, animal studies are currently predominant in establishing mechanisms and causality, whereas human studies are predominant in providing observational, epidemiological, and postmortem evidence of disease associations.

Oral-Gut Microbiota and Cognitive Impairment and Decline

Neural Pathways

Neurological disorders affect an estimated 15% of the global population, a figure projected to increase because of aging and growing environmental stressors.[15,16] Among these, neuropsychiatric conditions such as depression, schizophrenia, and autism spectrum disorder are particularly challenging because they are chronic, highly comorbid, and often resistant to conventional treatments.[1] Depression and anxiety alone affect approximately 15–20% of the population.[7]

Emerging research implicates the oral microbiome in cognitive function and mental health. Although individuals share a "core" oral microbiome, genetic, environmental, and lifestyle factors drive substantial interindividual variability.[17] One study by Adnan et al.[18] found that reduced cognitive function correlated with a higher prevalence of pro-inflammatory oral bacteria, including *Treponema*, *Parvimonas*, and *Filifactor*.[19] Whether these microbial shifts can serve as reliable biomarkers for cognitive decline remains unclear; however, the findings suggest that maintaining oral health or modulating the oral microbiome may help prevent or mitigate age-related cognitive deterioration, including Alzheimer's disease.[15,16]

Immune Pathways

Alzheimer's disease (AD), characterized by progressive cognitive decline, has been increasingly linked to chronic periodontitis. Research suggests that oral bacteria and their metabolic byproducts may reach the central nervous system (CNS) through either the bloodstream or peripheral nerves, where they may contribute to the development of neurodegenerative changes.[18] Key pathogens implicated in this process include *P. gingivalis*, *T. denticola*, *F. nucleatum*, *P. intermedia*, *A. actinomycetemcomitans*, and *Streptococcus species*. These microorganisms can produce virulence factors, such as lipopolysaccharides, gingipains, and peptidoglycans, which may cross the blood-brain barrier and provoke chronic neuroinflammation, one of the central mechanisms in AD pathology (Fig. 3).[11,17] Notably, postmortem studies have shown elevated levels of gingipains, produced by *P. gingivalis*, in the brains of individuals with AD compared with healthy controls.[16,20] The most abundant studies are postmortem studies, which show an association between certain microbes

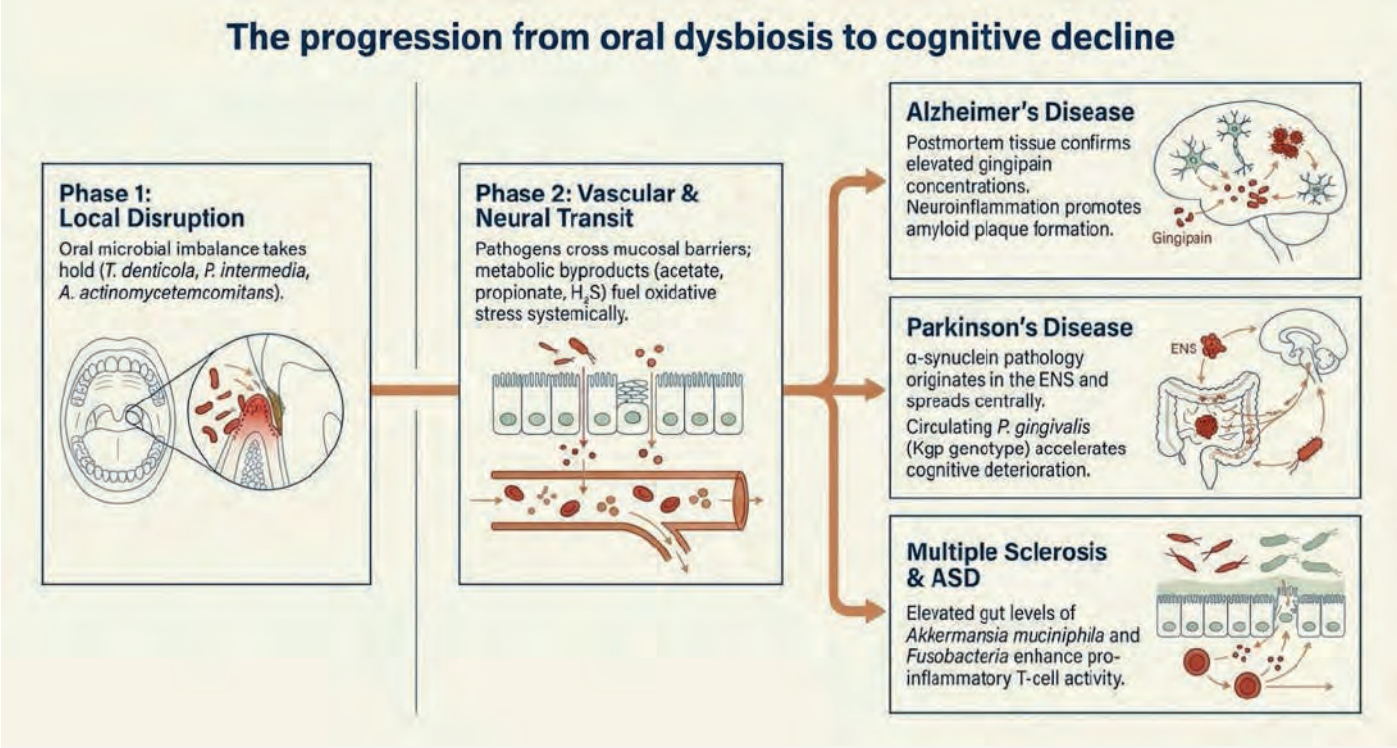

Figure 3. Progression from oral dysbiosis to cognitive decline.

and AD. Beyond direct microbial invasion, metabolites such as short-chain fatty acids, including acetate and propionate, and gases such as hydrogen sulfide (H_2S), produced during oral dysbiosis, may exacerbate neurodegeneration by promoting oxidative stress and inflammatory cascades.[14] Supporting this, experiments in mice in which saliva from patients with periodontitis was introduced into the gut led to intestinal inflammation and microbiota imbalances, suggesting a systemic effect of oral microbial imbalance.[17] To bridge this evidence gap, *P. gingivalis* and its toxins are currently being targeted in ongoing clinical trials, such as one evaluating a lysine-gingipain inhibitor, aiming to reduce its impact in patients with AD.

Microbial and Metabolic Pathways

Parkinson's disease (PD) also shows intriguing microbial associations, with α-synuclein pathology potentially originating in the enteric nervous system. Notably, up to 80% of patients with PD report constipation years before diagnosis. Circulating *P. gingivalis* has been detected in these individuals, with a specific Kgp genotype correlated with worsening cognitive symptoms.[6,21]

Multiple sclerosis (MS) and autism spectrum disorder (ASD) are similarly being linked to microbial imbalances. In MS, elevated levels of *Akkermansia muciniphila* and *Acinetobacter calcoaceticus* may enhance pro-inflammatory T-cell activity while reducing regulatory T-cell function. In addition, elevated gut *Fusobacteria* levels have been associated with future MS relapses.[22] Much of the current understanding of proposed causal relationships and mechanistic pathways is derived from animal studies, which limits direct extrapolation to humans. In contrast, human evidence is largely observational and demonstrates associations between oral pathologies and cognitive decline, along with altered microbial profiles in mild cognitive impairment and AD, although causal inference remains limited because of study design and potential confounding.

Oral Microbiota and Orofacial Pain

Neural Pathways

Orofacial pain, typically experienced in the face, jaw, or oral tissues, has traditionally been attributed to dental problems, muscular dysfunction, or nerve disorders. However, growing research indicates that the oral microbiome may play a more direct role in pain modulation by influencing both local and systemic inflammatory responses and nerve signaling.[23] Periodontitis serves as a prime example of how oral pathogens may contribute to pain. Bacteria such as *P.*

gingivalis, *T. denticola*, and *Tannerella forsythia* release harmful substances, including lipopolysaccharides, gingipains, and outer membrane vesicles. These components can disrupt immune balance and stimulate the release of inflammatory molecules, such as TNF-α, IL-1β, and prostaglandins, all of which increase sensitivity in local nerve endings and amplify pain perception. Specifically, *P. gingivalis* lipopolysaccharide can activate receptors such as TLR4 and TRPA1, which are found on sensory neurons of the trigeminal nerve, the main pain pathway for the orofacial region. Activation of these receptors has been associated with a cascade of inflammatory signals that heighten nerve activity and sensitivity.

Immune Pathways

The trigeminal nerve is not only critical for transmitting nociceptive signals but may also be susceptible to modulation by microbial products. Experimental studies, largely derived from *in vitro* and animal models, suggest that bacterial toxins and inflammatory mediators can stimulate neurons within the trigeminal ganglion, potentially inducing the release of neuropeptides such as substance P and calcitonin gene-related peptide.[10] These mediators are known to play a role in neurogenic inflammation and may contribute to pain persistence; however, direct causal evidence in humans remains limited. In chronic conditions such as burning mouth syndrome and idiopathic facial pain, alterations in the oral microbiome have been reported, but these findings are primarily associative. Consequently, although microbial dysbiosis may be implicated in the pathophysiology of these neuropathic pain conditions, the current evidence does not establish a definitive causal relationship, and further well-designed clinical studies are required. Another limitation is that studies use varied sampling and sequencing techniques, making it difficult to compare results across studies with small sample sizes.

Microbial and Metabolic Pathways

In addition, new evidence points to an important interaction between the oral and gut microbiomes. Certain oral bacteria, such as *F. nucleatum* and *P. gingivalis*, may migrate to the gut, where they disrupt the normal microbial environment, weaken the intestinal barrier, and provoke systemic inflammation. These inflammatory processes may extend to the nervous system through immune and hormonal pathways, potentially influencing brain function and increasing vulnerability to chronic pain and mood disorders. Although such mechanisms provide a plausible link between microbial imbalance and conditions such as temporomandibular disorders, which frequently coexist with anxiety and depression, current evidence is largely correlational and insufficient to establish a direct causal relationship.[23] In addition, long-term prospective studies tracking shifts in microbiome composition alongside the progression of orofacial pain are lacking.

Oral Health for a Healthy Oral-Gut-Brain Axis: Interventions

Diet

Diet has a substantial influence on oral microbial composition and its downstream effects on orofacial and systemic health. Diets high in refined carbohydrates and added sugars selectively favor acid-producing species, including *Actinomyces* spp. and *Streptococcus* mitis, which accelerate the development of dental caries and increase both local and systemic inflammatory burden, further worsening periodontal status. In contrast, dietary patterns characterized by high fiber content, whole grains, polyunsaturated fatty acids, and antioxidant-rich plant foods, as exemplified by the Dietary Approaches to Stop Hypertension (DASH) and Mediterranean dietary models, are associated with a substantially reduced risk of periodontal disease. An analysis of data from more than 6,200 adults found that close adherence to these dietary patterns was correlated with lower odds of periodontitis, whereas low intake of whole grains and dietary fiber was associated with a 32% and 27% higher likelihood of severe periodontitis, respectively.

Plant-based dietary patterns, including vegetarian and vegan diets, appear to confer periodontal benefits, likely because of their anti-inflammatory composition. However, potential reductions in calcium and vitamin B12 intake, as well as lower salivary pH, may increase susceptibility to enamel erosion and dental caries and should be considered in clinical counseling.[6,7]

Functional foods have attracted growing interest as adjuncts in periodontal management. These include mangosteen, a source of xanthone compounds, green tea, curcumin, and fermented lingonberry juice (FLJ). In a 1-year controlled human study, participants who rinsed daily with 10 mL of FLJ mouthwash for 30 seconds experienced significant improvements in salivary pH, buffering capacity, and flow rate, as well as improvements in clinical indicators, including bleeding on probing, plaque accumulation indexes, and microbiome composition.[24]

Probiotics and Prebiotics: Dosage and Regimen

Probiotics, defined by the World Health Organization as living microorganisms that, when administered in sufficient quantities, confer health benefits on the host, have demonstrated the capacity to modulate oral biofilm architecture, immune tone, and inflammatory activity. Clinical trial data indicate that lozenges containing *Lactobacillus reuteri* at a dose of 4×10^8 CFU per day, administered as one lozenge twice daily, over periods of

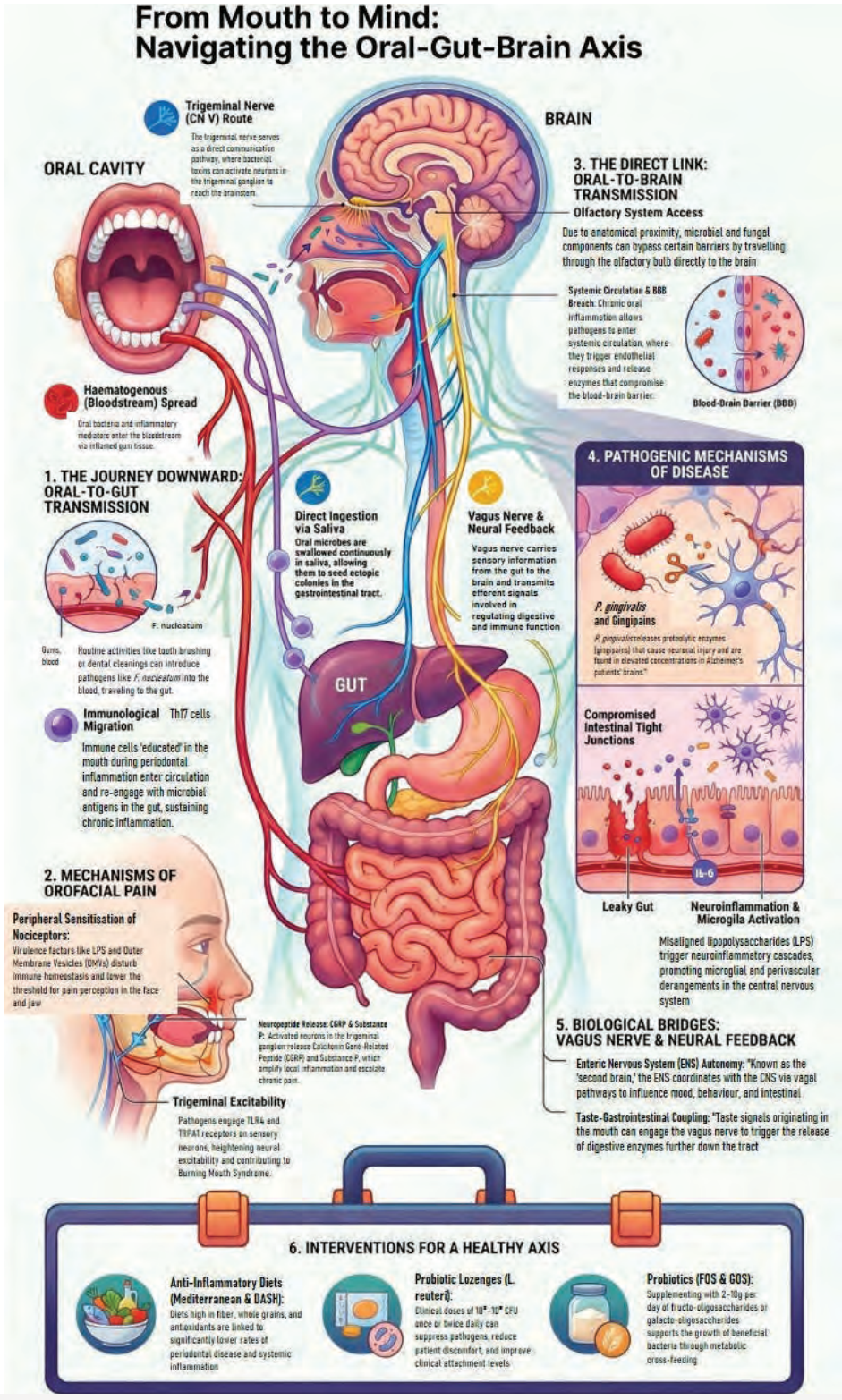

Figure 4. Summary of the article, illustrating how microbes travel from the oral cavity to the gut, how they interact with the gut, their role in neurodegenerative disorders, the possible mechanisms involved, and the interactions between the microbiome and the blood-brain barrier that contribute to the development of neurodegenerative disorders and orofacial pain.

Table 1. Key oral microorganisms, mechanisms, and clinical relevance in the oral-gut-brain axis

Key organism	Mechanism of action	Clinical relevance
Porphyromonas gingivalis	Produces gingipains and lipopolysaccharide; activates TLR4/TRPA1 on trigeminal neurons; disrupts the complement system; outer membrane vesicles transport virulence factors systemically; promotes blood-brain barrier disruption and neuroinflammation	Chronic periodontitis; implicated in Alzheimer's disease and Parkinson's disease
Treponema denticola	Sensitizes the trigeminal ganglion through inflammatory mediators; shows synergistic virulence with *P. gingivalis*	Periodontitis and orofacial pain; co-detected with *P. gingivalis* in postmortem brain tissue from patients with Alzheimer's disease; contributes to systemic inflammatory burden
Prevotella intermedia	Causes hemolysin-mediated cytotoxicity; collagenase degrades connective tissue; exhibits hormone-responsive growth related to estrogen and progesterone	Identified alongside *P. gingivalis* in postmortem studies of Alzheimer's disease; associated with adverse cerebrovascular outcomes
Fusobacterium nucleatum	FadA adhesin activates β-catenin/Wnt signaling; Fap2 suppresses natural killer/T-cell antitumor activity; disrupts enteric glial cells and gut barrier integrity	Colorectal cancer, with enrichment in tumor tissue and links to chemotherapy resistance; inflammatory bowel disease; neuroinflammation in preclinical models; elevated gut levels may predict multiple sclerosis relapses
Streptococcus mutans	Glucosyltransferases form the biofilm matrix; acid production demineralizes enamel; cnm-positive strains bind collagen and cross the blood-brain barrier	Dental caries; infective endocarditis; hemorrhagic stroke and cerebral microbleeds associated with cnm-positive strains

The associations listed do not imply causation. Much of the mechanistic evidence is derived from in vitro and animal studies; therefore, caution is warranted when extrapolating these findings to human clinical populations. TLR: Toll-like receptor; LPS: lipopolysaccharide; BBB: blood-brain barrier; OMV: outer membrane vesicle; AD: Alzheimer's disease; NK: natural killer cell; MS: multiple sclerosis.

3–12 weeks after scaling and root planing significantly reduce pocket depth, improve clinical attachment levels, lower plaque and bleeding indices, suppress key pathogens, including *P. gingivalis* and *A. actinomycetemcomitans*, and delay pathogen recolonization, with benefits maintained at 1-year follow-up. Comparable clinical improvements were achieved with *L. brevis* CD2 lozenges dosed at 1×10^8 CFU per day, with efficacy observed in both smoking and nonsmoking populations.

A recent randomized controlled trial used chewable tablets combining *L. salivarius*, *L. paracasei*, and *L. acidophilus* at 5×10^9 CFU per tablet taken once daily over 3 months, resulting in reductions in pocket depth and bleeding on probing, increased salivary IgA levels, and decreased *Fusobacterium* and *Porphyromonas* concentrations.[9]

Based on the available evidence, a recommended probiotic protocol would involve lozenge, tablet, or chewing gum delivery at a dosage of 10^8–10^9 CFU once or twice daily for 4–12 weeks, ideally administered alongside scaling and root planing, with continued maintenance use to prolong clinical benefit.

Prebiotics are defined, in line with the framework proposed by the International Scientific Association for Probiotics and Prebiotics in 2016, as substrates selectively metabolized by host microorganisms in ways that confer a health benefit.[6] Compounds in this category include inulin, fructooligosaccharides, galactooligosaccharides, resistant starches, and dietary polyphenols, each serving as a fermentable substrate that supports probiotic colonization and facilitates cross-feeding interactions among beneficial bacteria. Standard dosing ranges from 3–10 g per day for a minimum of 4–8 weeks. Synbiotic formulations combining probiotics and prebiotics in a single regimen may yield superior outcomes; however, clinicians should be aware that high prebiotic intake requires rigorous oral hygiene to reduce associated cariogenic risks.

In clinical applications aimed at improving health, prebiotics are most effectively used as adjuncts to probiotics within synbiotic regimens. Because certain prebiotic substrates are selectively fermented by particular bacterial taxa, this strategy can be used to deliberately support the establishment of targeted beneficial species. The metabolic byproducts of one

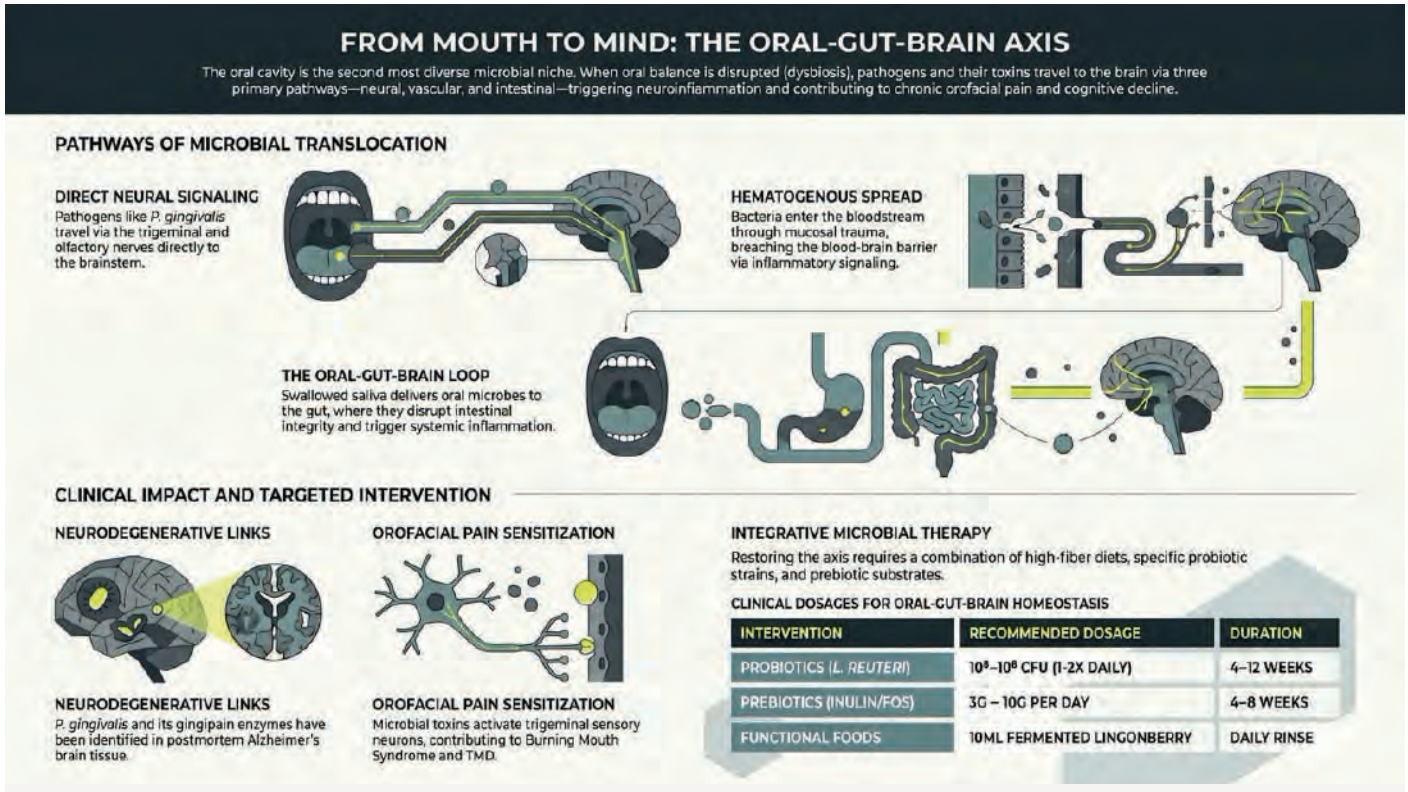

Figure 5. The three proposed pathways by which oral microbial dysbiosis may influence neurological and systemic health: **(a)** direct neural signaling, **(b)** hematogenous spread, and **(c)** the oral-gut-brain loop. The lower panel depicts the clinical associations and therapeutic implications of these pathways and integrative microbial therapy.

organism's fermentation activity may further serve as growth substrates for others, a cooperative metabolic process known as cross-feeding.[6]

Integrative Oral Care Approach

A comprehensive approach to oral health integrates dietary modification, evidence-based probiotic and prebiotic protocols, and consistent oral hygiene practices. Daily prebiotic supplementation in the range of 3–10 g, combined with probiotic lozenges dosed at 10^8–10^9 CFU and administered over a 4–12-week period, can meaningfully support oral microbial balance. Functional foods, including mangosteen and fermented lingonberry juice, provide complementary antioxidant and antimicrobial activity. These microbial interventions should be paired with standard hygiene practices, including twice-daily brushing, interdental cleaning, sugar-free gum use, and judicious mouthwash application. Broad-spectrum antimicrobial agents, such as chlorhexidine and triclosan, are clinically useful but may deplete beneficial microorganisms when used excessively; therefore, their use should be balanced with microbe-supportive strategies.

CONCLUSION

The intricate relationship among oral microbial communities, orofacial pain, and neurodegenerative disease is a rapidly advancing area of scientific inquiry with meaningful clinical implications. A growing body of evidence supports the view that oral dysbiosis is not merely a localized pathological process but a condition capable of contributing to systemic and neurological dysfunction through inflammatory, immunological, and neuroendocrine mechanisms. Figure 4 provides an illustrated and comprehensive overview of the various mechanisms and possible associations between the oral microbiome, neurodegenerative disorders, and orofacial pain. Pathogens such as *P. gingivalis* and *T. denticola* are implicated not only in periodontal disease but also in neuroinflammatory responses, blood-brain barrier compromise, and progressive cognitive decline. The trigeminal nerve, altered salivary microbial profiles, and the overarching oral-gut-brain axis together underscore the extensive systemic reach of oral microbial communities in shaping central pain processing and neurological outcomes. However, many of the mechanistic links identified thus far remain incompletely defined, and causal directionality has yet to be definitively established (Table 1).

There is an urgent need for rigorously designed longitudinal and interventional studies to clarify the specific pathways by which the oral microbiome contributes to orofacial and neurodegenerative pathology. Figure 5 provides an overview of interventions that could contribute to preventing the possible precipitation of the aforementioned diseases. Future research should prioritize the identification of microbial biomarkers capable of supporting early disease detection, the development of microbiome-targeted therapies, and the design of individualized oral care strategies with broader neurological benefits. As medicine moves toward a more integrated understanding of human biology, the oral microbiome may prove to be both a valuable diagnostic indicator and a modifiable therapeutic target in the prevention and management of chronic pain and neurocognitive disorders. Although animal models help clarify mechanisms, human studies are crucial to validate whether findings such as *P. gingivalis* invasion of the human brain represent clinically relevant processes rather than laboratory effects. The field is moving from proving association in humans to establishing causation in animal models and developing interventions.

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Comparison of the Effectiveness of Dexmedetomidine Sedation and General Anesthesia Following Interscalene Block in Proximal Humerus Fracture Surgery

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ABSTRACT

Objective: This study aimed to evaluate the effects of dexmedetomidine sedation and general anesthesia combined with ultrasound-guided interscalene brachial plexus block (ISB) on perioperative patient satisfaction, surgeon comfort, pain management, and length of hospital stay in the surgical management of proximal humeral fractures.

Materials and Methods: Patients who underwent surgical treatment for proximal humerus fractures between January 2021 and June 2024 were retrospectively evaluated. All procedures were performed following ISB, either in combination with dexmedetomidine sedation or general anesthesia. A total of 72 patients were included in the final analysis (Group I: ISB with dexmedetomidine sedation, n=35; Group II: ISB with general anesthesia, n=37). Baseline demographic and clinical characteristics were comparable between the groups. Perioperative outcomes, including the patient satisfaction score (PSS) and surgeon comfort score (SCS), were assessed using 10-point numerical rating scales. Additional outcomes included postoperative pain scores, opioid consumption, motor function recovery, complications, and length of hospital stay.

Results: The PSS was significantly higher in Group I, whereas the SCS was significantly higher in Group II (both $p<0.001$). Intraoperative hypotension and postoperative nausea and vomiting occurred more frequently in Group II than in Group I, with statistically significant differences between the groups ($p<0.05$). In contrast, no significant intergroup differences were observed in perioperative numerical rating scale scores, total systemic analgesic consumption, time to recovery of motor strength, or length of hospital stay ($p>0.05$).

Conclusion: ISB combined with sedation provided greater patient satisfaction and fewer perioperative complications, whereas general anesthesia improved surgeon comfort. These findings suggest that ISB with sedation is a preferable approach for patients undergoing shoulder surgery.

Keywords: Dexmedetomidine sedation, general anesthesia, interscalene brachial plexus block, patient satisfaction, proximal humerus fracture, surgeon comfort.

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INTRODUCTION

Among skeletal injuries, proximal humerus fractures account for nearly 10% of all fractures and constitute a significant proportion of upper-extremity trauma, with an incidence that increases with age.[1,2] Proximal humerus fractures cause significant pain and functional impairment in affected patients.[3,4] The use of regional anesthesia (RA), most notably interscalene brachial plexus block (ISB), has gained prominence as a primary method for postoperative pain management in shoulder surgery.[3] This approach improves patient satisfaction in the postoperative period while reducing opioid consumption and associated complication rates.[3,5,6] Because shoulder surgery can cause severe perioperative pain and certain complications, an optimal anesthetic method should be selected. Multimodal analgesia is used in perioperative pain management and includes central and peripheral nerve blocks in addition to various pharmacologic agents. Nevertheless, because opioid use is often associated with a range of adverse outcomes, including nausea, vomiting, delirium, constipation, and respiratory depression, regional anesthesia methods have become increasingly preferred.[7,8] In addition to effective analgesia, patient and surgeon comfort are also important in anesthesia management. In recent years, RA techniques, especially ISB, have been preferred to provide effective shoulder analgesia while allowing the patient to remain conscious during surgery.[9,10] Although the COVID-19 pandemic accelerated the adoption of regional anesthesia techniques, the preference for regional approaches in shoulder surgery has persisted beyond the pandemic period because of their advantages in reducing perioperative complications, minimizing airway manipulation, and improving recovery profiles.[11,12]

RA techniques in shoulder surgery have predominantly been used in combination with general anesthesia (GA) to enhance perioperative analgesia and patient satisfaction. However, data on the outcomes of proximal humerus fracture surgery performed exclusively under RA, without GA, remain scarce. To the best of our knowledge, no previous study has specifically evaluated the perioperative outcomes of proximal humerus fracture fixation performed solely under ISB, as applied in the present study.[13,14]

Thus, this study was designed to evaluate the effects of two anesthetic strategies—dexmedetomidine sedation with ultrasound-guided ISB and general anesthesia with ultrasound-guided ISB—on perioperative patient satisfaction, surgeon comfort, pain management, and length of hospital stay in patients undergoing proximal humerus fracture fixation.

KEY MESSAGES

- Dexmedetomidine sedation following ISB significantly increased patient satisfaction, whereas the combination of general anesthesia with ISB improved surgeon comfort.
- Sedation was associated with lower rates of postoperative nausea and vomiting and hypotension, whereas pain scores, opioid consumption, and length of stay were comparable between the groups.
- The findings support regional-first anesthesia strategies, particularly during extraordinary periods such as the COVID-19 pandemic.

MATERIALS AND METHODS

Study Setting and Design

This study was conducted at a state hospital affiliated with the Ministry of Health. The medical records of patients who underwent surgical treatment for proximal humerus fractures between January 2021 and June 2024 were reviewed. The study was designed to compare perioperative outcomes between two anesthetic strategies: ultrasound-guided interscalene brachial plexus block (ISB) combined with dexmedetomidine sedation and ISB combined with general anesthesia.

Ethics Approval

Ethical approval was obtained from the Bilecik Seyh Edebali University Clinical Research Ethics Committee (Approval Number: 317242, Date: 10.03.2025). All research procedures complied with institutional ethical standards and the principles of the Declaration of Helsinki.

Patients and Data Collection

The diagnostic criteria included patients who underwent surgery for proximal humerus fractures between January 2021 and June 2024. Patients aged 18 to 90 years who underwent ISB for the surgical management of proximal humerus fractures were considered eligible for inclusion. The exclusion criteria were defined as follows:

1. Any history of prior surgical procedures involving the same shoulder.
2. Fractures resulting from pathological bone disease.
3. Patients who could not undergo ISB because of local anesthetic allergy, coagulopathy, anticoagulant therapy, infection at the block site, a history of neurologic deficit in the upper extremity scheduled for surgery, or inability to communicate.
4. Patients with insufficient data.

Patients were enrolled consecutively. Allocation to the anesthetic groups was not randomized but was based on

institutional practice and patient preference. During the COVID-19 period, regional anesthesia with sedation was recommended when feasible; however, general anesthesia was performed if preferred by the patient after appropriate preoperative counseling. Perioperative data for all patients were routinely recorded during clinical care using structured evaluation forms by an independent assessor who was not involved in anesthesia management or surgical procedures. These data included pain intensity, patient satisfaction, surgeon comfort, analgesic use, and length of hospital stay. For the purposes of this study, all recorded data were retrospectively retrieved from hospital records and analyzed. Patients included in the study were divided into two groups: Group I consisted of patients who underwent sedation with dexmedetomidine after ISB, and Group II consisted of patients who underwent general anesthesia after ISB.

Block and Surgical Procedure

All patients were monitored with standard electrocardiography, noninvasive blood pressure measurement, and pulse oximetry upon arrival in the recovery room. Ultrasound-guided ISB was performed by an experienced anesthesiologist (A.G.) using a high-frequency linear probe under strict aseptic conditions, with the patient in a semi-lateral position. After the brachial plexus was identified between the anterior and middle scalene muscles, a 21-gauge needle was advanced in-plane, and 20 mL of local anesthetic solution (10 mL of 0.5% bupivacaine + 10 mL of 2% lidocaine) was administered with intermittent aspiration (Fig. 1). Sensory and motor block adequacy was confirmed, and the patients were transferred to the operating room approximately 30 minutes later.

In the sedation group, sedation depth was clinically monitored using the Ramsay Sedation Scale at regular intraoperative intervals, and dexmedetomidine infusion (0.2–0.7 µg/kg/h) was titrated to maintain the target sedation level (score 3). However, detailed time-based sedation records were not available because of the retrospective design. In the general anesthesia group, anesthetic induction was achieved with propofol, fentanyl, and rocuronium, followed by intubation. Anesthesia was maintained with age-adjusted 1 MAC sevoflurane, and ventilation parameters were adjusted to maintain end-tidal CO_2 between 35 and 40 mmHg. Short-acting IV opioids were permitted intraoperatively if hemodynamic changes exceeded 20% from baseline.

The operations were performed at a single center by a single surgeon (M.G.) with 5 years of experience in trauma surgery. All patients were placed in the beach-chair position, and standard sterile preparation was performed. A deltopectoral approach was used to access the proximal humerus. Hematoma and soft-tissue interposition were cleared, and traction sutures were

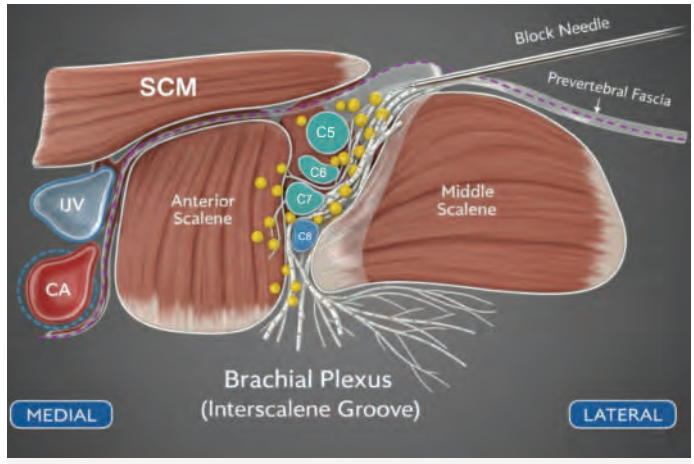

Figure 1. Ultrasound anatomy for interscalene brachial plexus block.

SCM: Sternocleidomastoid muscle; IJV: Internal jugular vein; CA: Common carotid artery; C5: Fifth cervical nerve; C6: Sixth cervical nerve; C7: Seventh cervical nerve; C8: Eighth cervical nerve.

placed when necessary to mobilize displaced tuberosities. Anatomic reduction was achieved under fluoroscopic guidance, with restoration of the medial calcar. Provisional K-wire fixation was followed by application of a precontoured locking proximal humerus plate lateral to the bicipital groove. Final fluoroscopic images confirmed implant positioning and screw length before layered closure.

Postoperative Follow-up Procedure

The primary outcomes were the patient satisfaction score and surgeon comfort score. The secondary outcomes were pain at 1, 6, 12, 18, and 24 hours postoperatively, assessed using the numeric rating scale (NRS); first-hour and total systemic analgesic consumption; motor function assessment; length of hospitalization; and perioperative or postoperative complications.

Patient satisfaction and surgeon comfort were assessed using 10-point numerical rating scales. Surgeon comfort was recorded on a scale of 1 to 10 (1=very uncomfortable, 10=very comfortable). Patient satisfaction was recorded 24 hours postoperatively on a scale of 1 to 10 (1=poor, 10=excellent).

Patients received postoperative analgesia according to the standard protocol. Intravenous acetaminophen 1 g was administered at 6-hour intervals and withheld if the NRS score was ≤2. When the NRS score was ≥4, intravenous tramadol 50 mg was administered as rescue analgesia; if pain persisted after 30 minutes, an additional 50 mg was administered (maximum: 100 mg). If analgesia remained inadequate,

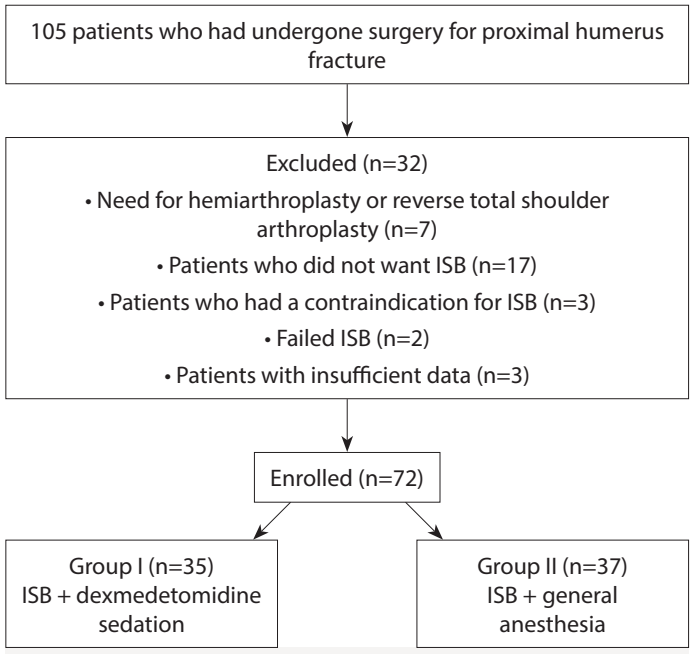

Figure 2. Presentation of the patients included in the study according to the inclusion and exclusion criteria.

ISB: Interscalene block.

intravenous morphine 3 mg was administered as second-line rescue analgesia. Time to first rescue analgesia and total analgesic requirement were recorded. Motor function was assessed using a modified Bromage scale adapted for the upper extremity.

Intraoperative hypotension was defined as a decrease in mean arterial pressure of >20% from baseline or to below 65 mmHg. PONV was defined as nausea, retching, or vomiting within 24 hours postoperatively. Ondansetron was administered as required according to the institutional protocol. In Group I, two patients required conversion to general anesthesia because of inadequate analgesia and were excluded from the final analysis.

Statistical Analysis

The normality of quantitative variables was assessed using the one-sample Shapiro-Wilk test. Categorical variables were presented as frequencies and percentages, normally distributed continuous variables as means with standard deviations, and non-normally distributed variables as medians with minimum–maximum ranges. Between-group comparisons of categorical variables were performed using the chi-square test. For continuous variables, Student's t-test was used when normality assumptions were met, whereas the Mann–Whitney U test was used for non-normally distributed data.

Because no established minimal clinically important difference (MCID) values exist for patient satisfaction or surgeon comfort scores, which are single-item, nonvalidated satisfaction measures, an anchor-based MCID calculation was not feasible. Therefore, a distribution-based approach was adopted, and the MCID was defined as 0.5 standard deviation of the pooled scores, a commonly accepted statistical method for estimating clinically meaningful differences in such outcomes. In addition, a Patient Acceptable Symptom State (PASS) analysis was performed to support clinical interpretability, with scores ≥8 on the 10-point scale considered indicative of high patient satisfaction and surgeon comfort. No additional multivariable adjustment was performed because of the retrospective design and relatively limited sample size; however, baseline demographic and clinical characteristics were comparable between the groups.

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), Version 20.0 (SPSS Inc., Chicago, IL, USA). A p-value of <0.05 was considered statistically significant.

RESULTS

Among the 105 patients initially assessed for eligibility, 33 were excluded based on the predefined exclusion criteria. Consequently, 72 patients constituted the final study population. Of these, 35 patients were assigned to Group I and 37 to Group II (Fig. 2). The mean age of the patients was 57.7 years (range, 22–79 years). The sex distribution was comparable, comprising 35 female and 37 male patients. The distribution of fracture patterns according to the Arbeitsgemeinschaft für Osteosynthesefragen (AO) classification was comparable between the two groups, with no statistically significant difference observed, indicating a balanced fracture severity profile across the groups. The duration of surgery was also comparable between the two groups, with no statistically significant difference observed. No significant intergroup differences were observed in baseline demographic variables. The descriptive demographic and clinical characteristics of the study cohort are presented in Table 1.

Functional Results

The median patient satisfaction score was 9 (6–10) in Group I and 8 (6–10) in Group II. The patient satisfaction score was significantly higher in Group I (p<0.001) (Table 2).

The median surgeon comfort score was 8 (5–10) in Group I and 9 (5–10) in Group II. The surgeon comfort score was significantly lower in Group I (p<0.001) (Table 2). The surgeon comfort score was assessed by a single experienced surgeon, which may have introduced a degree of subjectivity into the evaluation.

Table 1. Baseline demographic and clinical characteristics of the study population

Variables	Group I (n=35)	Group II (n=37)	p
Age, years	59 (24–79)	56.5 (22–79)	0.14[a]
Sex, male/female, n (%)	17–18 (48.5–51.5)	20–17 (54–46)	0.12[b]
Fracture type, n (%)			0.12[b]
AO 11A1	5	6	
AO 11A2	8	8	
AO 11A3	8	7	
AO 11B1	7	8	
AO 11B2	3	3	
AO 11B3	3	3	
AO 11C1	2	1	
AO 11C2	1	1	
AO 11C3	0	0	
Operation duration, min	52.5 (45–60)	51.5 (43–60)	0.47[b]
BMI, kg/m^2	27.18 (19.48–31.25)	26.68 (21.99–31.64)	0.16[a]
ASA class, I/II/III, n (%)	4–21–10 (11–60–29)	5–22–10 (13.5–59.5–27)	0.32[b]
No comorbid diseases, n (%)	14 (40)	23 (62)	0.18[b]
Hypertension, n (%)	27 (77)	24 (64)	0.44[b]
Diabetes mellitus, n (%)	27 (77)	27 (72)	0.79[b]
Thyroid disease, n (%)	2 (6)	3 (8)	0.83[b]
Smoking, n (%)	12 (34)	13 (35)	0.95

Data are presented as median (minimum–maximum) unless otherwise indicated. a: Mann–Whitney U test; b: Chi-Square Test. AO: Arbeitsgemeinschaft für Osteosynthesefragen; ASA: American Society of Anesthesiologists; BMI: body mass index.

The incidence of postoperative nausea, vomiting, and intraoperative hypotension was significantly higher in Group II than in Group I (p=0.01, p=0.01, and p=0.02, respectively) (Table 2).

No statistically significant differences were observed between the groups in terms of postoperative NRS scores, time to first opioid use, total opioid dose, or length of hospital stay (p>0.05) (Table 2, 3).

A total of 72 patients underwent ISB (ISB+D: n=35; ISB+GA: n=37). No life-threatening complications were observed in either group. Hoarseness developed in two patients in Group I and completely resolved during inpatient follow-up.

The mean difference in patient satisfaction scores between the groups was 1.01 points. Based on a pooled standard deviation of 1.08, the calculated MCID was 0.54. Because the observed difference exceeded the MCID threshold, the difference was considered clinically meaningful. Similarly, the mean difference in surgeon comfort scores was 1.32 points, with a pooled standard deviation of 1.06, yielding an MCID of 0.53. The observed intergroup difference also exceeded the MCID threshold. PASS analysis demonstrated that patient satisfaction scores in Group I consistently exceeded the predefined PASS threshold (≥8), whereas surgeon comfort scores more frequently reached PASS in the general anesthesia group (Table 4).

DISCUSSION

In this study, patient satisfaction among patients undergoing proximal humerus fracture surgery was higher in the dexmedetomidine sedation group after ISB, whereas the surgeon comfort score was higher in the general anesthesia group after ISB. The incidence of intraoperative hypotension and postoperative nausea and vomiting was higher in the general anesthesia group than in the dexmedetomidine group. Neither group was superior with respect to postoperative pain scores, total opioid dose, time to first analgesic requirement, or length of hospital stay.

Various techniques, such as arthroscopy, open surgery, and joint replacement, are commonly used to treat shoulder pathologies. The choice of anesthesia for shoulder surgery is important for

Table 2. Comparison of perioperative patient outcomes between the groups

Variables	Group I (n=35)	Group II (n=37)	p
PSS	9 (6–10)	8 (6–10)	<0.001[a*]
SCS	8 (5–10)	9 (5–10)	<0.001[a*]
Intraoperative hypotension, yes/no, n (%)	5/30 (14–86)	10–27 (27–73)	0.02[b*]
Intraoperative hypertension, yes/no, n (%)	8/27 (27–73)	9–28 (25–75)	0.26[b]
Surgical duration, min	110 (90–140)	110 (90–140)	0.82[a]
Time to first opioid use, h	11.5 (9–16)	10.5 (9–16)	0.26[a]
Total postoperative opioid consumption, mg tramadol hydrochloride	50 (0–100)	50 (0–100)	0.21[a]
Duration of motor block, h	8 (5–9)	6.5 (5–10)	0.38[a]
Postoperative nausea, n (%)	10 (28)	18 (48.6)	0.01[b*]
Postoperative vomiting, n (%)	2 (5.7)	11 (28)	0.01[b*]
Length of hospital stay, days	2 (1–4)	2 (1–5)	0.11[b]

Data are presented as median (minimum–maximum) unless otherwise indicated. a: Mann–Whitney U test; b: Chi-Square Test; *: Statistically significant. PSS: patient satisfaction score; SCS: Surgeon comfort score.

ensuring patient comfort, optimizing surgical conditions, and minimizing postoperative pain.[15] Multimodal analgesia aims to optimize postoperative pain management by targeting different receptors in pain pathways with systemic analgesics, such as opioids, and techniques such as nerve blocks.[7,16] Opioid-sparing or opioid-free anesthesia, which aims to avoid opioid-related side effects, is mainly achieved using peripheral or central nerve block techniques. Several blocks, such as ISB, supraclavicular block, suprascapular block, and intra-articular injections, are used for perioperative pain control in shoulder surgery. In particular, ISB has been reported to provide long-lasting and effective postoperative pain control and to reduce opioid-related side effects by decreasing opioid consumption.[7,17] Although ISB is most commonly used in combination with general anesthesia to provide postoperative analgesia for shoulder surgery, it can also be used alone for shoulder surgery. Compared with general anesthesia, ISB has been reported to increase patient comfort and satisfaction.[18,19] Studies on the use of ISB alone or in combination with general anesthesia have reported conflicting results.[20,21] ISB is often combined with general anesthesia to take advantage of the benefits of both techniques. However, the potential side effects of both techniques may also increase.[20,22]

Table 3. Intergroup comparison of perioperative pain scores at rest and during movement

Variables	Group I (n=35)	Group II (n=37)	p
rNRS in the recovery room	1 (0–2)	1 (0–2)	0.17
mNRS in the recovery room	1 (0–3)	1 (0–2)	0.08
rNRS at postoperative 2 h	1 (0–2)	1 (0–2)	0.09
mNRS at postoperative 2 h	2 (0–3)	2 (0–3)	0.26
rNRS at postoperative 6 h	1 (0–3)	2 (0–4)	0.19
mNRS at postoperative 6 h	3 (0–5)	3 (1–7)	0.13
rNRS at postoperative 12 h	2 (0–5)	2 (0–4)	0.13
mNRS at postoperative 12 h	3 (0–7)	3 (1–7)	0.29
rNRS at postoperative 18 h	2 (1–4)	2 (1–3)	0.13
mNRS at postoperative 18 h	3 (2–6)	3 (2–6)	0.30
rNRS at postoperative 24 h	1 (0–3)	1 (0–3)	0.26
mNRS at postoperative 24 h	3 (1–4)	3 (1–4)	0.10

Data are presented as median (minimum–maximum) unless otherwise indicated. Mann–Whitney U test. mNRS: Numerical rating scale score during movement; rNRS: Numerical rating scale score at rest.

One of the most important steps in modern hospital quality management is assessing and improving perioperative patient satisfaction.[22] Patient satisfaction is influenced by many factors, such as postoperative complications, nausea, vomiting, itching, early mobilization, and early return to daily physical activities, in addition to perioperative pain.[20–22] Many studies have shown that assessing patient satisfaction and maintaining it at the highest possible level are integral components of optimal anesthesia management.[20,22] Garip et al.[23] reported that dexmedetomidine sedation increased patient satisfaction and decreased postoperative complications, such as hoarseness and sore throat, compared with general anesthesia in dental surgery. Similarly, the current study showed that dexmedetomidine sedation after ISB in proximal humerus fracture surgery increased patient satisfaction and decreased postoperative complications compared with general anesthesia. This may be because general anesthesia is more invasive, may increase complications such as nausea, vomiting, chills, and sore throat, and may be associated

Table 4. Clinical relevance of patient satisfaction and surgeon comfort scores based on MCID and PASS analyses

Variables	Group I (n=35)	Group II (n=37)	Pooled SD	MCID (0.5 SD)	Exceeds MCID	PASS threshold
PSS	9 (6–10)	8 (6–10)	1.08	0.54	Yes	Yes (Group I)
SCS	8 (5–10)	9 (5–10)	1.06	0.53	Yes	Yes (Group II)

Data are presented as median (minimum–maximum) unless otherwise indicated. The minimal clinically important difference was calculated using a distribution-based method (0.5×pooled SD). The Patient Acceptable Symptom State was defined as a score ≥8 on a 10-point scale. MCID: Minimal clinically important difference; PASS: Patient Acceptable Symptom State; PSS: Patient satisfaction score; SCS: Surgeon comfort score; SD: Standard deviation.

with patient anxiety regarding general anesthesia procedures.[23] In addition, the current study found that the surgeon comfort score was higher with ISB+general anesthesia than with ISB+dexmedetomidine sedation. This may be because complete muscle relaxation under general anesthesia facilitates surgical manipulation and intervention in the surgical field.

Postoperative nausea and vomiting (PONV) not only impair patient comfort but can also affect patient recovery and early discharge.[24,25] Opioid anesthesia is known to be more strongly associated with PONV than nonopioid anesthesia.[26] In addition, inhaled anesthetics have also been shown to be strongly associated with PONV.[27] Therefore, reducing the use of inhaled anesthetics and opioids may reduce PONV. In the current study, patients in the dexmedetomidine group experienced less PONV. These results may be related to the use of sevoflurane to maintain general anesthesia, opioid use, and procedures such as intubation attempts.[24,25] In addition, intraoperative hypotension was less common in patients in the dexmedetomidine group in this study. This may be due to the various techniques used in general anesthesia, including intravenous and inhaled anesthetics, and their effects on hemodynamic reflex mechanisms.

With advances in ultrasound technology, interest in peripheral nerve blocks has increased in recent years. Ultrasound allows the practitioner to visualize the needle in real time, enabling more successful blocks with lower doses of local anesthetic and reducing the risk of vascular, nerve, or soft-tissue damage.[7] Although some studies suggest that ISB does not increase the risk of peripheral nerve injury, it should be noted that it can cause permanent or temporary nerve damage.[28] ISB can cause phrenic nerve block, which may lead to Horner syndrome and severe respiratory failure.[29] Several studies have investigated how to minimize pulmonary dysfunction associated with ISB, but the risk has not been completely eliminated.[7] In the present study, no clinically significant respiratory complications were observed. However, two patients in the dexmedetomidine group developed transient hoarseness after ISB, which resolved completely during ward follow-up.

Many studies have investigated the choice of local anesthetic and the administered volume. Fredrickson et al.[30] reported that 20 mL of 0.375% ropivacaine provided analgesia similar to that of 30 mL of 0.5% ropivacaine in shoulder surgery but increased patient satisfaction. Riazi et al.[31] reported that the use of a 5-mL volume of local anesthetic in ultrasound-guided ISB resulted in similar postoperative analgesia and fewer respiratory and other complications compared with 20 mL. However, Gautier et al.[32] demonstrated that although successful blocks could be achieved with 5 mL of 0.75% ropivacaine in ultrasound-guided ISB, the failure rate was high (25%). Different local anesthetic mixtures can be used to regulate the onset and duration of the block.[33] In the present study, successful ISB was performed with a mixture of 10 mL of 0.5% bupivacaine and 10 mL of 2% lidocaine. Studies have reported ISB success rates of 84%–96% using the nerve stimulator technique for shoulder surgery.[20,34] Another study reported a 97.5% success rate for ultrasound-guided ISB, similar to that in our study.[20]

In recent years, increasing emphasis has been placed on distinguishing statistical significance from clinical relevance in perioperative outcome research. While minimal clinically important difference (MCID) thresholds have been well established for validated, multi-item patient-reported outcome measures, such as the American Shoulder and Elbow Surgeons (ASES) and Constant scores, no consensus exists regarding MCID values for single-item, satisfaction-based measures, including patient satisfaction and surgeon comfort scores.[35,36] These outcomes are frequently reported in anesthesia and perioperative medicine studies; however, they are typically interpreted using absolute score differences rather than clinically meaningful thresholds, limiting their interpretability across studies.[37,38] To address this methodological limitation, we applied a distribution-based MCID estimation using 0.5 standard deviation of the pooled scores, a widely accepted and recommended approach when anchor-based MCID calculation is not feasible.[39] This method has been extensively used in clinical research to approximate meaningful change in single-item or non-validated outcome measures and allows for a standardized interpretation of between-group differences beyond statistical significance alone. In the present study, the observed intergroup differences in both patient satisfaction and surgeon comfort scores exceeded the calculated MCID thresholds, supporting the clinical

relevance of our findings. In addition, we complemented the distribution-based MCID analysis with a Patient Acceptable Symptom State (PASS) approach to further enhance clinical interpretability. PASS represents a patient-centered concept reflecting a threshold beyond which patients consider their condition satisfactory, and it has been increasingly used in orthopedic and perioperative research as an anchor for meaningful outcomes.[40] By defining PASS as a score ≥8 on the 10-point scale, we were able to contextualize satisfaction outcomes in terms of clinically acceptable states rather than numerical differences alone. The concordance between MCID exceedance and PASS achievement in our cohort strengthens the robustness of our conclusions and provides a more comprehensive assessment of patient- and surgeon-centered outcomes. Taken together, this combined approach offers a pragmatic and methodologically sound framework for interpreting satisfaction-based outcomes in shoulder surgery anesthesia studies.

This study has several limitations. Detailed intraoperative sedation depth recordings were not systematically documented because of the retrospective design. Subgroup analyses based on fracture severity were not performed. The surgeon comfort score was assessed using a non-validated single-item scale by a single surgeon, which may have introduced subjective bias. Although no clinically significant respiratory complications were observed, respiratory outcomes, including hemidiaphragmatic paralysis, were not objectively evaluated, representing an important limitation given the known phrenic nerve involvement associated with interscalene block.

Despite these limitations, this study has several strengths. All interscalene blocks were administered by the same anesthesiologist, and all surgeries were performed by a single surgeon using standardized techniques, minimizing variability. To our knowledge, this is the first study to estimate MCID values for patient satisfaction and surgeon comfort scores in proximal humerus fracture surgery. Additionally, perioperative assessments were performed by an independent nurse, reducing observational bias.

CONCLUSION

In conclusion, dexmedetomidine sedation following interscalene block was associated with higher patient satisfaction and fewer perioperative complications, whereas general anesthesia was associated with improved surgeon comfort. However, given the retrospective design and inherent limitations of the study, these findings should be interpreted with caution. Future prospective, randomized studies with standardized outcome measures are needed to confirm these results and better define the optimal anesthetic approach for proximal humerus fracture surgery.

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Thirteen-Year Clinical Outcomes Following Arthroscopic Resection of Symptomatic Mediopatellar Plica with Cartilage Degeneration

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ABSTRACT

Objective: This study aimed to evaluate the 13-year clinical and functional outcomes of arthroscopic removal of symptomatic mediopatellar plica (MPP) in patients with cartilage degeneration.

Materials and Methods: Seventy-six patients who underwent arthroscopic MPP excision between 2005 and 2006 were retrospectively reviewed. After exclusions and loss to follow-up, 42 patients with a mean age of 51.2±12.1 years were evaluated at a mean of 14 years postoperatively. Clinical outcomes were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) at baseline, 6 months, and final follow-up. Symptoms, plica types, and coexisting intra-articular pathologies were also recorded.

Results: A total of 42 patients with a mean age of 51.2±12.1 years were evaluated at a mean follow-up of 14.0±0.79 years. Significant improvements in pain and function were observed at 6 months and at final follow-up ($p<0.05$). At 6 months, 97.6% of patients rated their outcome as good or excellent according to WOMAC scores, and 92.9% maintained this rating at final follow-up. Improvements were consistent regardless of the presence of additional intra-articular pathology. No patient reported worsening of symptoms during follow-up. Cartilage degeneration was observed in all cases, predominantly in the medial femoral condyle and medial pole of the patella.

Conclusion: Arthroscopic excision of symptomatic MPP associated with cartilage degeneration provides durable clinical benefits lasting more than a decade. The sustained improvement in pain and function supports arthroscopic plica resection as an effective treatment option when conservative therapies fail, even in patients with coexisting joint pathology.

Keywords: Arthroscopy, cartilage degeneration, knee joint pathology, long-term outcomes, medial plica, synovial plica syndrome.

INTRODUCTION

The medial synovial plica develops from mesenchymal tissue during embryogenesis and remains as a vestigial structure within the knee joint. Epidemiological studies have shown that it is present in approximately 64% to 84% of the general population.[1,2] The plica is a flexible structure in the knee that usually does not restrict movement. Inflammation and swelling may result from various causes, including trauma or noninjury-related factors, often associated with patellofemoral joint function. Loss of plica flexibility may lead to cartilage damage and symptoms of medial plica syndrome (MPS). Many patients with anterior knee pain (AKP) have a thickened medial plica; however, the main contributing factors remain unclear.[3]

The mediopatellar plica (MPP) is the most common synovial plica in the knee and is a frequent cause of pain, particularly in younger patients.[4] A recent study reported an incidence rate of 79.9%, indicating that this condition is common across age groups. Symptomatic cases are more frequent in young and active individuals, with a slightly higher prevalence among males, possibly due to activity-related factors.[5] Nonspecific symptoms, such as edema, instability, a sensation of blocking, false locking, and clicking sounds, may occur, even when pain is intermittent and triggered by activities such as stair climbing.[5] Moreover, previous studies have reported that cartilage degeneration accompanies symptomatic MPP in approximately 24% to 50% of cases, most frequently involving the medial femoral condyle and the medial pole of the patella.[6,7] The initial phase of treatment may be conservative. However, arthroscopic excision of the inflamed plica should be considered if it crosses the medial femoral condyle and has damaged the patellofemoral joint cartilage.[1,8] Multiple studies have supported arthroscopic removal as an effective treatment for persistent MPP symptoms that do not improve with conservative methods.[5,9–11] However, limited information is available on the long-term clinical outcomes after arthroscopic treatment.[6]

This study focused on evaluating the long-term clinical outcomes following MPP excision. The hypothesis was that surgical removal of MPP would result in significant improvements in patient well-being and favorable clinical outcomes.

METHODS

This retrospective cohort study evaluated the long-term clinical outcomes of arthroscopic excision of symptomatic MPP. The long-term findings of a previously published clinical cohort were included in the present study.[5] Arthroscopic plica excision was performed in 76 patients between 2005 and 2006, with a minimum follow-up of 13 years. The study was approved by the Erciyes University Clinical Research Ethics Committee (Approval Number: 2023/206; Date: 29.03.2023), and all included patients provided written informed consent. Eligible patients had symptomatic MPP and MRI-confirmed cartilage deterioration at the medial femoral condyle or medial patella that had not improved after 6 months of conservative treatment (Fig. 1). Patients with osteoarthritis, previous knee surgery, angular deformities, joint laxity, or rheumatological disorders were excluded.[5] During long-term follow-up, patients were re-evaluated by phone and invited to the clinic. Approximately 15 years after surgery, no information could be obtained from the hospital system for 10 patients. After patient selection for long-term follow-up, one patient who had undergone knee replacement surgery after total

KEY MESSAGES

- Arthroscopic mediopatellar plica excision provides durable pain relief and functional improvement over 14 years.
- Favorable outcomes can be achieved even in the presence of concomitant cartilage degeneration and additional intra-articular pathology.
- Plica morphology alone does not appear to determine long-term clinical prognosis after arthroscopic resection.

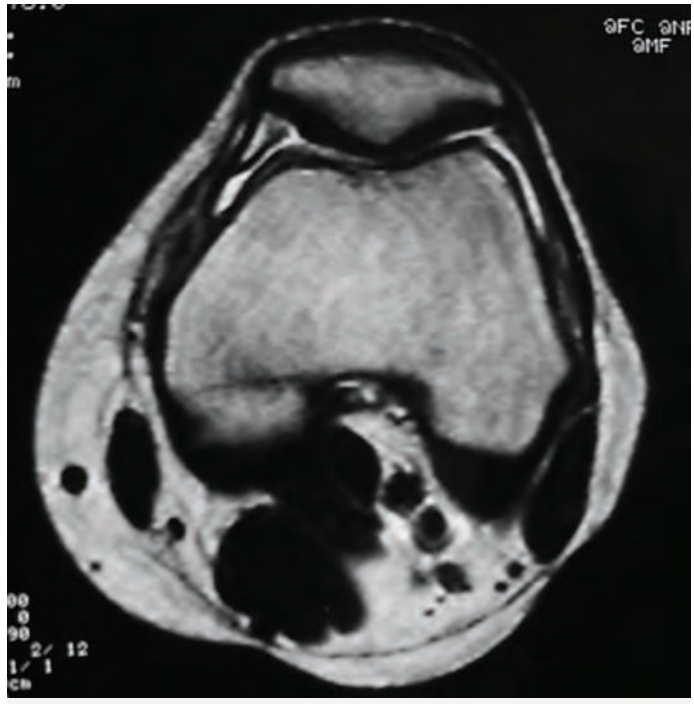

Figure 1. Preoperative MRI showing the mediopatellar plica.

meniscectomy, one patient who had undergone ipsilateral knee trauma surgery, two patients who subsequently underwent meniscectomy, and 20 patients who were lost to follow-up were excluded. Of the 20 patients lost to follow-up, 13 could not be reached using the phone number registered in the system, and seven were contacted but declined further contact. Finally, 42 patients who underwent arthroscopic resection of MPP of the knee were retrospectively evaluated after at least 13 years.

Surgical Procedures

The patients underwent surgery under either regional or general anesthesia. All arthroscopic procedures were performed by the same surgical team under the supervision of a senior orthopedic surgeon, ensuring a standardized operative technique. Standard anterolateral (AL) and anteromedial (AM) portals were used during the surgical intervention. A thorough examination of all knee compartments, including the MPP and other intra-articular pathologies, was performed, and the intraoperative appearance of the mediopatellar plica was documented during arthroscopy (Fig. 2). First, pathologies other than MPP were evaluated. The mediopatellar plica was classified according to the Dandy classification, which categorizes synovial plicae based on their width relative to the suprapatellar pouch and their structural characteristics.[2]

According to this system, the plica may be absent (Type A), have a width of up to one-quarter of the suprapatellar pouch (Type B), extend between one-quarter and one-third of the pouch (Type C), extend between one-third and two-thirds of the pouch (Type D), or exceed two-thirds of the pouch width (Type E). Additional morphological variants include a complete membrane (Type F), a perforated membrane (Type G), an arch configuration (Type H), a pillar configuration (Type I), and a lateral plica (Type J). This classification was applied intraoperatively to evaluate plica morphology and its potential mechanical impact on the medial femoral condyle. Medial and lateral meniscal tears were repaired, and patients with anterior cruciate ligament (ACL) rupture underwent ACL reconstruction. In this study, no degenerative or diffuse cartilage lesions were observed. Microfracture was performed only for focal full-thickness cartilage defects smaller than 2 cm^2 located in weight-bearing areas. Debridement was performed in patients with synovial and Hoffa hypertrophy. Next, from the perspective of the AL portal, a motorized shaver was inserted through the AM portal. The MPP was then partially excised, leaving a thin, circular margin along its periphery while avoiding injury to the capsule and synovium. A drain was placed at the end of the operation, the incisions were sutured, and the operated extremity was wrapped up to the proximal one-third of the thigh using 15-cm-wide elastic bandages.

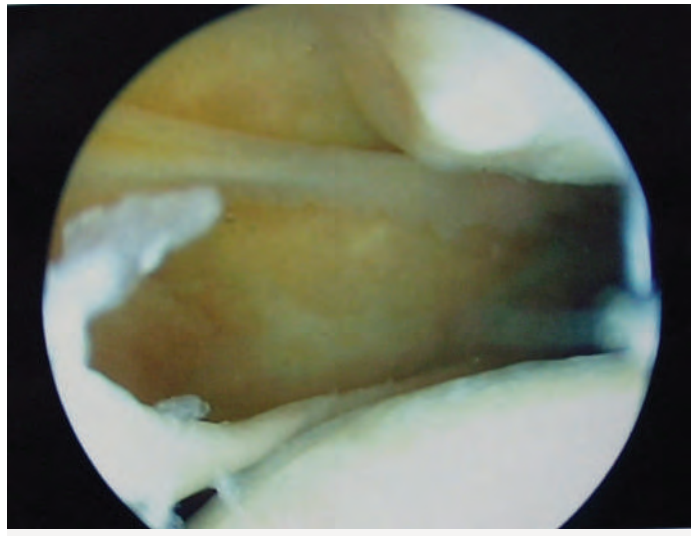

Figure 2. Intraoperative arthroscopic view of the mediopatellar plica.

Follow-up and Rehabilitation

After the procedure, cold compression was applied, and the drains were removed the following day. One week later, the sutures were removed, and patients continued to receive medical care along with an exercise program. For patients with meniscal tears, the postoperative rehabilitation plan depended on the location and severity of the tear. In general, patients were allowed to mobilize without weight-bearing support for 3 weeks. From weeks 3 to 6, patients were allowed partial weight bearing with a brace, and full weight bearing was permitted after 6 weeks. Moderate activities, such as light jogging, were permitted 3 months after surgery, and sports activities were resumed after 6 months. Physical therapy began on postoperative day 1 and included limited range-of-motion exercises: 45° flexion until week 4, followed by 90° flexion until week 6, and then progression to knee-strengthening exercises. Patients who underwent isolated ACL reconstruction began isometric quadriceps, patellar mobilization, and hamstring exercises on day 1. Range-of-motion exercises were started immediately to achieve 0° to 90° knee ROM within the first week. Once patients achieved 0° to 100° ROM and demonstrated muscle control, full weight bearing was permitted. Patients who underwent microfracture were allowed to mobilize without weight bearing for 3 weeks, and exercises to preserve joint range of motion began on the first day after surgery. Partial weight bearing was permitted from weeks 3 to 6, with full weight bearing beginning after week 6. For the remaining patients, ROM exercises began on postoperative day 1 without restrictions, and full weight bearing was permitted.[5]

Assessments

In the initial study, comprehensive patient records were meticulously documented preoperatively, including patient characteristics, detailed medical histories, and findings from thorough physical examinations. In addition, additional pathologies and joint degeneration were recorded during preoperative MRI and arthroscopic surgery. Cartilage degeneration was evaluated and graded according to the Outerbridge classification. Preoperatively, postoperatively, at 6 months, and 13 years later, patients were specifically asked about pain patterns, the relationship between pain and activity, the cinema sign, and false locking. A knee examination was routinely performed, particularly around the medial pole and medial joint space, to assess tenderness. Preoperative conventional radiographs, including anteroposterior and lateral views, and MRI were performed in all patients. Pain levels and physical function during daily activities were assessed using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire, which consists of 24 questions. The pain score ranged from 0 to 28, and the physical function score ranged from 0 to 63. The possible mean WOMAC score ranged from 0 to 3.83. The mean WOMAC score was used for the clinical and functional evaluation of patients before and after surgery, and the results were categorized as follows: 0 to 0.75, excellent; 0.75 to 1.75, good; 1.75 to 2.75, moderate; and >2.75, worse.

Statistical Analyses

All statistical analyses were performed using SPSS 22.0 for Windows (SPSS Inc., IL, USA). Descriptive statistics included the mean, standard deviation, median, minimum and maximum values, and frequency ratios. The Kolmogorov-Smirnov test was used to assess the distribution of variables. For independent quantitative variables, either the independent-samples t-test or the Mann-Whitney U test was used, depending on whether the data were normally distributed. The Wilcoxon test was used for paired quantitative data. Because the assumptions of the Pearson chi-square test were violated due to small expected cell frequencies, Fisher's exact test was used for categorical comparisons, including the analysis of plica type distributions between groups. Cochran's Q test was used to compare dependent dichotomous data across multiple time points. The Bonferroni adjustment was applied with the McNemar test to identify differences between time points. The Friedman test was used to compare data across more than 2 time points, followed by Bonferroni-corrected Wilcoxon rank tests for pairwise comparisons. Statistical significance was set at $p<0.05$. Mean±standard deviation values were also reported to facilitate comparison with previous studies.

Table 1. Demographic and clinical characteristics of the patients

Characteristics	Total (n=42)
Age (years)	51.2±12.1
BMI (kg/m^2)	27.5±4.1
Sex, n (%)	
Female	17 (40.5)
Male	25 (59.5)
Side, n (%)	
Right	21 (50.0)
Left	21 (50.0)
Medial meniscal injury, n (%)	
(–)	19 (45.2)
(+)	23 (54.8)
Lateral meniscus injury, n (%)	
(–)	35 (83.3)
(+)	7 (16.7)
ACL rupture, n (%)	
(–)	37 (88.1)
(+)	5 (11.9)
Hoffa hypertrophy, n (%)	
(–)	35 (83.3)
(+)	7 (16.7)
Patellofemoral discordance, n (%)	
(–)	39 (92.9)
(+)	3 (7.1)
Cartilage defect, n (%)	
(–)	34 (81.0)
(+)	8 (19.0)
Synovial hypertrophy, n (%)	
(–)	40 (95.2)
(+)	2 (4.8)
Follow-up duration, median (min–max), years	14 (13–15)
Time from symptom onset to surgery, median (min–max), months	12.5 (7–32)

BMI: body mass index; ACL: anterior cruciate ligament; (+): present; (–): absent.

RESULTS

A total of 42 patients (17 females and 25 males) with a mean age of 51.2±12.1 years were included in the final evaluation. The mean follow-up duration was 14.0±0.79 years. Detailed demographic and clinical characteristics are presented in Table 1. Of all patients, 11 had isolated MPP, whereas the remaining

Table 2. Patient characteristics and plica types according to additional pathology status

Characteristics	Isolated MPP plica (n=11)	MPP + additional pathologies (n=31)	p
Age, mean±SD, years	46.9±13.5	52.7±11.4	0.172
BMI, mean±SD, kg/m²	27.3±4.2	27.2±4.2	0.962
Sex, n (%)			0.746
Female	4 (36.4)	13 (41.9)	
Male	7 (63.6)	18 (58.1)	
Side, n (%)			0.726
Right	5 (45.5)	16 (51.6)	
Left	6 (54.5)	15 (48.4)	
Follow-up duration, median (min–max), years	14 (13–15)	14 (13–15)	0.412
Time from symptom onset to surgery, median (min–max), months	10 (7–32)	13.4 (7–25)	0.592
Plica type, n (%)			
Type B	0 (0)	0 (0)	–
Type C	4 (36.4)	9 (29.0)	0.457
Type D	2 (18.2)	16 (51.6)	0.080
Type E	1 (9.1)	1 (3.2)	0.467
Type F	0 (0)	2 (6.5)	0.545
Type G	0 (0)	0 (0)	–
Type H	4 (36.4)	3 (9.7)	0.063

Data are expressed as n (%), mean±SD, or median (min–max). Plica type comparisons were performed using Fisher's exact test. BMI: Body mass index; MPP: Mediopatellar plica; SD: Standard deviation.

31 had MPP in conjunction with at least one additional condition. According to the Dandy classification, Type D plica appeared to be more frequent in patients with additional pathologies than in those with isolated MPP; however, this difference did not reach statistical significance according to Fisher's exact test (51.6% vs. 18.2%, p=0.080). Similarly, Type H plica appeared to be more frequent in patients with isolated MPP than in those with additional pathologies, but this association was also borderline and not statistically significant (36.4% vs. 9.7%, p=0.063). Therefore, these findings were interpreted with caution and were not considered statistically significant. The demographic and clinical characteristics and plica types according to the presence of additional pathology were comparable between the groups, as shown in Table 2.

Cartilage degeneration of at least grade 1 was present in all patients at both the medial femoral condyle and the medial pole of the patella. Grade ≥2 lesions were observed in 90.5% of medial femoral condyles and 85.7% of medial patellar poles (Table 3). All signs and symptoms showed significant reductions in frequency at both the 6-month and final follow-up evaluations compared with baseline. In addition, the WOMAC total and subgroup scores showed significant improvement at both early and long-term follow-up compared with baseline, with early-term results being significantly superior to long-term outcomes (Table 4).

Table 3. Distribution of cartilage degeneration by grade and location

Grade	Medial femoral condyle n (%)	Medial pole of the patella n (%)
Grade 1	4 (9.5)	6 (14.3)
Grade 2	22 (52.4)	20 (47.6)
Grade 3	15 (35.7)	14 (33.3)
Grade 4	1 (2.4)	2 (4.8)
Total	42 (100.0)	42 (100.0)

Most patients rated their outcomes as good or excellent based on their mean WOMAC scores (97.6% at the 6-month follow-up and 92.9% at the final follow-up). During the follow-up period, none of the patients reported worsening of outcomes. Subgroup analysis showed that signs and symptoms improved similarly in patients with and without additional diseases. At final follow-up, WOMAC pain, physical function, and total

Table 4. Changes in clinical features and WOMAC scores throughout the study

Characteristics	Preoperative	At 6 months	At final follow-up
Pain, n (%)	42 (100)	5 (11.9)[a]	9 (21.4)[b]
Intermittent pain, n (%)	41 (97.6)	3 (7.1)[a]	8 (19.0)[b]
Pain on activity, n (%)	40 (95.2)	4 (9.5)[a]	9 (21.4)[b]
Pain when climbing stairs, n (%)	42 (100)	5 (11.9)[a]	8 (19.0)[b]
Pain when squatting, n (%)	40 (95.2)	4 (9.5)[a]	8 (19.0)[b]
Cinema sign, n (%)	40 (95.2)	1 (2.4)[a]	6 (14.3)[b]
Stiffness during initial steps, n (%)	35 (83.3)	3 (7.1)[a]	5 (11.9)[b]
False locking, n (%)	26 (61.9)	2 (4.8)[a]	7 (16.7)[b]
Swelling, n (%)	22 (52.4)	1 (2.4)[a]	3 (7.1)[b]
Tenderness over the medial pole of the patella, n (%)	30 (71.4)	0 (0)[a]	3 (7.1)[b]
Tenderness over the medial joint space, n (%)	35 (83.3)	2 (4.8)[a]	5 (11.9)[b]
WOMAC scores			
Pain score, mean±SD/median (range)	8.0±3.8 / 8 (0–16)	0.8±2.5[a] / 0 (0–12)	3.7±4.2[bc] / 2 (0–15)
Physical function score, mean±SD/median (range)	25.2±11.3 / 27 (5–52)	2.7±7.9[a] / 0 (0–44)	8.8±11.7[bc] / 3.5 (0–45)
Total score, mean±SD/median (range)	33.5±14.3 / 33 (7–64)	3.6±10.4[a] / 0 (0–55)	15±15.7[bc] / 9 (0–60)
Mean score, mean±SD/median (range)	1.3±0.5 / 1.38 (0.29–2.67)	0.15±0.43[a] / 0 (0–2.29)	0.6±0.62[bc] / 0.36 (0–2.4)
Rating based on the mean WOMAC score			
Excellent, n (%)	–	40 (95.2)	27 (64.3)
Good, n (%)	–	1 (2.4)	12 (28.6)
Moderate, n (%)	–	1 (2.4)	3 (7.1)
Worse, n (%)	–	–	–

a: P<0.017. 6 months versus baseline; b: P<0.017, final follow-up versus baseline; c: P<0.017. 6 months versus last follow-up. Bonferroni correction was used. WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index; SD: Standard deviation.

scores remained significantly improved compared with baseline, although they were slightly higher than the 6-month values. No intraoperative or postoperative complications, including infection, hemarthrosis, instability, progression to patellofemoral osteoarthritis, or reoperation, were observed during long-term follow-up.

DISCUSSION

The key finding of this study was the notable clinical improvement observed over a 13-year follow-up period after surgical removal of MPP compared with the preoperative condition. Furthermore, this study is among those with the longest follow-up periods in this field. Although plicae are frequently detected during arthroscopy, plicae around the knee are rarely considered problematic. However, plica syndrome can significantly reduce a patient's quality of life and cause anterior knee pain. Inflammatory processes may lead to thickening and fibrotic changes in the plica, which may then act as a tightened band and impinge on the surrounding cartilage, contributing to joint damage.[12] Kang et al.[3] reported that the AKP group had a significantly higher frequency of MPP. They concluded that abnormalities in the patellofemoral (PF) joint, particularly MPP and minor irregularities in articular geometry, should be thoroughly assessed in patients with atraumatic AKP.

Dandy et al.[2] reported that MPP is present in most normal knees (64%–84%), but most symptomatic cases, accounting for 84.5% of patients, are associated with Type C and D plicae. Types A and B rarely cause pain, and when they do, conservative treatment and physiotherapy provide better outcomes than those observed for Type C and D plicae.[13,14] In another study, more than 80% of knees were classified as Type C or D.[15] A study of 3,563 patients and 3,889 knees reported a medial synovial plica incidence of 79.9%. Among these cases, Types A, B, C, and D accounted for 35.2%, 22.4%, 12.3%, and 10.0%, respectively.[16] In our study, Type C and D plicae constituted the majority of cases (73.9%), and these patients did not respond to conservative treatment for 6 months. The difference between our study and the study by

Nakayama et al.[16] may be explained by differences in the study populations; the previous study included only patients who underwent arthroscopic knee surgery. In contrast, our study included patients with medial plica syndrome who did not improve after 6 months of conservative treatment. Therefore, we believe that this study supports the existing literature. The symptomatology was consistent with that reported in previous publications. Arthroscopic plica excision was performed in all patients, and positive and significant outcomes were obtained in all cases.

Consistent with existing research, the initial approach usually involves conservative treatment, such as physiotherapy, activity restriction, and physical exercises, which have demonstrated favorable outcomes.[1,17] However, arthroscopic intervention may be necessary in patients with persistent pain. Recent literature has shown excellent outcomes after arthroscopic intervention in such cases; however, there are currently no definitive indications for selecting between conservative and surgical treatment.[1,6,17–20]

Blanke et al.[21] conducted a study in which patient-reported outcomes were evaluated using the Lysholm score, Visual Analog Scale (VAS), and Tegner activity score. The results showed excellent outcomes both in patients who underwent successful conservative treatment and in those who underwent surgery after failed conservative treatment. No statistically significant differences were observed between the two groups. Patients who underwent surgical treatment had a relatively low overall complication rate of 6.5%. In the present study, patients were evaluated using the WOMAC scoring system at 6 months after surgery and after a mean follow-up of 13 years. Nearly all patients reported excellent outcomes at the 6-month follow-up. WOMAC scores differed significantly between 6 months and 13 years. Similar clinical improvement was observed in both patients with isolated MPP and those with additional pathologies. Although WOMAC scores were better at 6 months, long-term follow-up showed excellent scores in 64.3% of patients, followed by good scores in 28.6% and moderate scores in 7.1%. Although our long-term results demonstrated that symptoms did not worsen over time, this interpretation should be made cautiously, as long-term radiological imaging, including MRI or X-ray, was not routinely available for this cohort. Therefore, the stability of symptoms over a 13-year period may reflect clinical improvement rather than documented structural preservation. The observed decline in clinical scores compared with early postoperative outcomes could be attributed to age-related physiological joint degeneration, progression of baseline chondral pathology, and reduced activity levels over time. These factors are likely to influence long-term functional capacity independently of the initial MPP pathology.

Paczesny et al.[6] reported that patients without cartilage abnormalities had better clinical outcomes after arthroscopic plica excision. The authors suggested that impaired neuromuscular control may cause abnormal patellar tracking, irritating the MPP and accelerating cartilage degeneration. They also emphasized the need for more controlled studies to determine whether physiotherapy or plica resection is appropriate at this clinical stage. Christophorakis and Strachan reported that 24.7% of 319 MPP cases among 1,000 patients showed cartilage degeneration in the medial femoral condyle and/or medial pole of the patella.[22] In our study, cartilage degeneration was found in all patients at the medial femoral condyle and medial pole of the patella, with grade ≥2 lesions observed in 90.5% and 85.7% of these sites, respectively. Cultural practices in this group, including activities such as squatting and sitting on the floor, may increase the risk of cartilage degeneration.[5]

A recent meta-analysis found that arthroscopic removal of symptomatic medial knee plica yielded satisfactory clinical outcomes in most cases. Therefore, arthroscopic excision should be considered a treatment option for patients who do not respond to initial nonsurgical interventions.[9]

In many studies, isolated MPP lesions were excised, and favorable outcomes were reported.[6,18] Patients with additional pathological conditions were included in this study, with no differences observed in age, BMI, sex, follow-up duration, or duration of preoperative symptoms between patients with isolated MPP and those with other pathologies. Early results indicated excellent outcomes for nearly all patients, as reflected by WOMAC scores.[5] At the final follow-up, conducted after a mean of 13 years, patients' preoperative scores showed significant improvement. The results of our study are consistent with previous findings reported in studies investigating plica excision.[23–28] Clinical improvements may have resulted from the management of coexisting conditions that responded to treatment. However, patients with isolated plica syndrome showed progress similar to that of patients with additional pathologies, with few exceptions. More controlled studies are needed to assess the long-term decline in clinical scores after a mean follow-up of 13 years.

The strengths of this study include the 13-year follow-up, thorough assessment of plica pathology, and treatment of additional conditions. The findings support the effectiveness of plica excision in improving outcomes in both isolated plica syndrome and other knee conditions. Future research should include controlled studies to investigate the long-term effects of plica excision and the factors influencing the decline in clinical scores over time. However, certain limitations remain. The small sample size limits the generalizability of the findings, primarily

because of the single-center design and the challenges of reaching participants after a mean follow-up of 13 years. In addition, the long follow-up period resulted in significant loss to follow-up, potentially introducing bias. Another key limitation is the absence of a control group, which prevents comparative conclusions. Although subgroup analyses were conducted between patients with isolated MPP and those with additional pathologies, these analyses cannot fully replace a true comparator group. Postoperative rehabilitation protocols also varied among patients who underwent additional procedures, such as meniscal repair, ACL reconstruction, or microfracture; however, the small number of patients in these subgroups made it impossible to statistically evaluate the impact of these differences on outcomes. Furthermore, long-term radiological imaging, including MRI or X-ray, was not available, which limits the interpretation of the finding that symptoms did not worsen over time, particularly in the context of loss to follow-up. The slight decline observed between early and long-term clinical outcomes may be attributed to age-related joint degeneration, progression of underlying chondral pathology, or changes in activity levels over the 13-year period. In addition, only the WOMAC score was used for functional assessment; other widely used functional or quality-of-life measures, such as the Lysholm score, IKDC score, and SF-36, were not available because they were not routinely documented during the original study period. Future studies should address these limitations by conducting multicenter research with larger patient cohorts and an appropriate control group to strengthen the validity and generalizability of the results.

CONCLUSION

When conservative treatments are ineffective, arthroscopic plica excision has demonstrated notable improvements in both the short and long term. This study supports the notion that arthroscopic excision is effective in preventing further damage to the patellofemoral joint and is therefore recommended for patients with symptomatic MPP.

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Janus Kinase Inhibitors in Atopic Dermatitis: A Global Bibliometric Analysis (2015–2025)

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ABSTRACT

Objective: In recent years, the therapeutic use of Janus kinase (JAK) inhibitors in dermatology, particularly in the management of atopic dermatitis (AD), has expanded markedly. This bibliometric study aimed to characterize research themes, evaluate the scientific productivity of authors and countries, and identify emerging areas that may guide future research in this field over the past decade.

Materials and Methods: A bibliometric analysis of JAK inhibitors in AD from 2015 to 2025 was conducted using the Web of Science Core Collection (WoSCC) database as the data source. Bibliometric analyses and data visualizations were performed using CiteSpace, VOSviewer, and the Bibliometrix package in R.

Results: A total of 1249 studies were included in the analysis. The cluster plot classified all keywords into 9 categories. Pruritus and alopecia areata (AA) were the most frequently co-occurring keywords alongside atopic dermatitis, while delgocitinib and abrocitinib were the most frequently investigated JAK inhibitors. The United States produced the highest number of publications over the past decade. Oregon Health and Science University emerged as one of the most significant contributors to global collaborations, while the Icahn School of Medicine had the highest number of publications, reaching approximately 175 by 2025. Simpson EL emerged as both the most influential and the most cited author in the field, as reflected by citation impact, followed by Emma Guttman-Yassky.

Conclusion: Janus kinase (JAK) inhibitors represent one of the most effective and trending therapeutic classes in AD. These agents are not only highly effective for AD but also show promising efficacy in other dermatologic conditions, such as AA.

Keywords: Atopic dermatitis, bibliometric, eczema, JAK, Janus kinase inhibitors.

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INTRODUCTION

Atopic dermatitis (AD), also known as atopic eczema, is a chronic inflammatory skin disorder characterized by recurrent eczematous lesions, xerosis, and persistent pruritus.[1] Its global

prevalence has increased progressively over recent decades, particularly in developed countries, affecting approximately 10%-20% of children and 2%-3% of adults worldwide.[2,3] In 2021, global pediatric AD cases reached 72.4 million, representing a 6.2% increase from 2000, with projections for 2024 estimating approximately 19.8 million affected adults and 9.2 million children in the United States (US) alone.[4,5] In many patients, disease onset occurs in early childhood and may persist into adulthood, resulting in a substantial long-term burden for both patients and healthcare systems.[6] Pruritus, a hallmark symptom of AD, is one of the most debilitating aspects of the disease and contributes significantly to sleep disturbance, psychological distress, and impaired quality of life.[7]

The pathogenesis of AD is complex and multifactorial, involving interactions among genetic predisposition, skin barrier dysfunction, immune dysregulation, and environmental factors.[8] The Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling pathway plays a central role in the pathogenesis of AD.[9] This pathway mediates the effects of various cytokines, such as interleukin (IL)-4, IL-13, and IL-31, which are critically involved in the immune response, pruritus, and inflammation in AD.[10] Specifically, JAK1 has been identified as a key factor in the inflammatory cascade of AD.[11]

In recent years, Janus kinase (JAK) inhibitors have emerged as a promising therapeutic strategy in dermatology, particularly for the management of moderate-to-severe AD.[12] By targeting the JAK-STAT signaling pathway, these agents simultaneously inhibit multiple cytokines involved in AD pathogenesis, resulting in rapid improvement in symptoms such as pruritus and skin inflammation.[13,14] Several JAK inhibitors, including baricitinib, upadacitinib, and abrocitinib, have received regulatory approval for AD, transforming the therapeutic landscape for patients who do not respond to conventional treatments.[15,16]

Despite the rapid expansion of the literature on JAK inhibitors in AD, a comprehensive evaluation of global research output and thematic trends in this field is needed. To our knowledge, this is the first systematic bibliometric analysis of research trends on JAK inhibitors in AD. Bibliometrics is a comprehensive knowledge system that integrates mathematics, statistics, and linguistics, with a primary focus on quantification. In the current era of big data, bibliometric approaches enable scientists and clinicians to delineate the research landscape and emerging hot spots within a given field, predict future research trends, and substantially improve research efficiency.[17]

The present study aimed to perform a global bibliometric analysis of publications on JAK inhibitors in AD over the past decade. Specifically, it sought to characterize publication trends, identify leading authors, institutions, and countries, and delineate the evolution of key research themes. Through this approach, the study aimed to inform both current clinical practice and future research directions in the field of AD therapeutics.

KEY MESSAGES

- Bibliometric analyses indicate that AD research has shifted from studies on skin barrier dysfunction and immunopathogenesis to randomized clinical trials of targeted therapies, particularly JAK inhibitors.
- The United States led scientific output and international collaboration, while Simpson EL and Guttman-Yassky E. were key contributors who shaped JAK inhibitor research in AD.
- Evidence supports the therapeutic potential of JAK inhibition in AD and other immune-mediated dermatologic diseases, particularly alopecia areata, reflecting shared inflammatory pathways and broader translational applications.

MATERIALS AND METHODS

Data Sources and Search Strategy

A bibliometric analysis of publications on JAK inhibitors in AD published from 2015 to 2025 was conducted using the Web of Science Core Collection (WoSCC) database as the primary data source. The search strategy was defined as follows: TS=("atopic dermatitis" OR "atopic eczema") AND TS=("Janus kinase inhibitor*" OR "JAK inhibitor*" OR baricitinib OR upadacitinib OR tofacitinib OR abrocitinib OR ruxolitinib OR delgocitinib OR itacitinib OR oclacitinib OR brepocitinib). The search was limited to articles published between 2015-01-01 and 2025-12-31. A total of 2,036 records were initially identified through the database search. Duplicate screening revealed no duplicate records. Title and abstract screening was performed to assess eligibility. Publications other than original articles and review articles were excluded, as were non-English publications and publications published in 2026. Following the screening process, 1,249 publications met the eligibility criteria and were included in the bibliometric analysis (Fig. 1). Ethical approval was not required for this study. The study was based exclusively on previously published articles. No patient-level data, clinical interventions, surveys, or experimental procedures were conducted.

Statistical Analysis

CiteSpace is citation analysis software developed in the field of scientometrics to identify research trends and visualize scientific knowledge structures. VOSviewer is a specialized

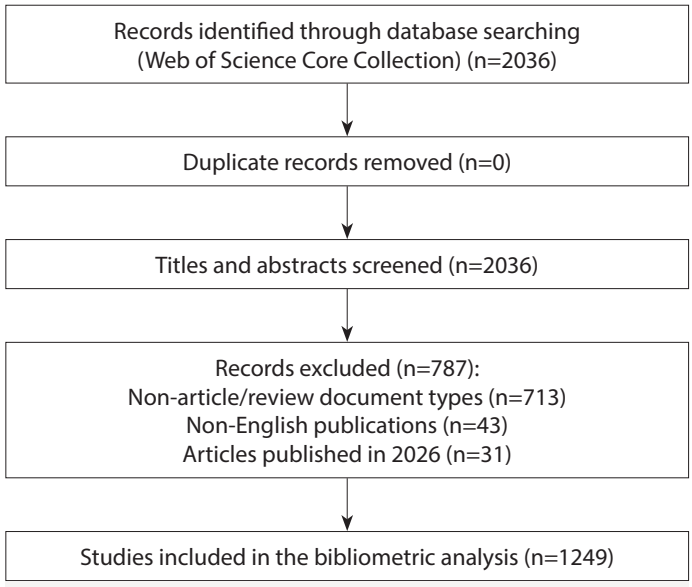


Figure 1. PRISMA flow diagram.

tool for constructing and visualizing bibliometric networks, including co-authorship, co-citation, and keyword co-occurrence maps. Bibliometrix is an advanced R package (version 5.0.1) that provides comprehensive bibliometric analysis and visualization capabilities. In this study, CiteSpace (version 6.3.R1) and the Bibliometrix R package were used to analyze authorship patterns, institutional and national contributions, journal distribution, reference networks, and keyword trends among the included articles. VOSviewer (version 1.6.20) was further used to perform co-occurrence and cluster analyses and to generate graphical visualizations of the bibliometric networks.

RESULTS

The initial search strategy yielded 2,036 records. After duplicate screening and study selection, 1,249 studies were included in the analysis. The analysis covered 337 sources, including journals and book chapters, from 2015 to 2025, with an average annual publication growth rate of 44.09%, indicating a marked increase in scientific production and growing academic interest in the field over time. The dataset included 6,157 authors, and 33 articles were written by a single author. International collaborations were present among 30.66% of the authors, while the average number of authors per article was 8.25, indicating that publications in this field were generally conducted by large teams and interdisciplinary partnerships. The number of publications on JAK inhibitors in patients with AD increased markedly after 2021. A total of 1,922 author keywords and 31,417 references were identified. The average number of citations per article was 29.75, and the life cycle of a paper was approximately 3.24 years. This metric represents the average duration between the publication year and the point at which citation frequency begins to decline significantly. Mathematically, it is derived from the mean citation age of documents within the dataset. This indicates that publications in the field attract substantial scholarly attention for a limited period and gradually lose influence over time. This finding also suggests that the research area is dynamic and rapidly evolving.

Authors and Affiliations

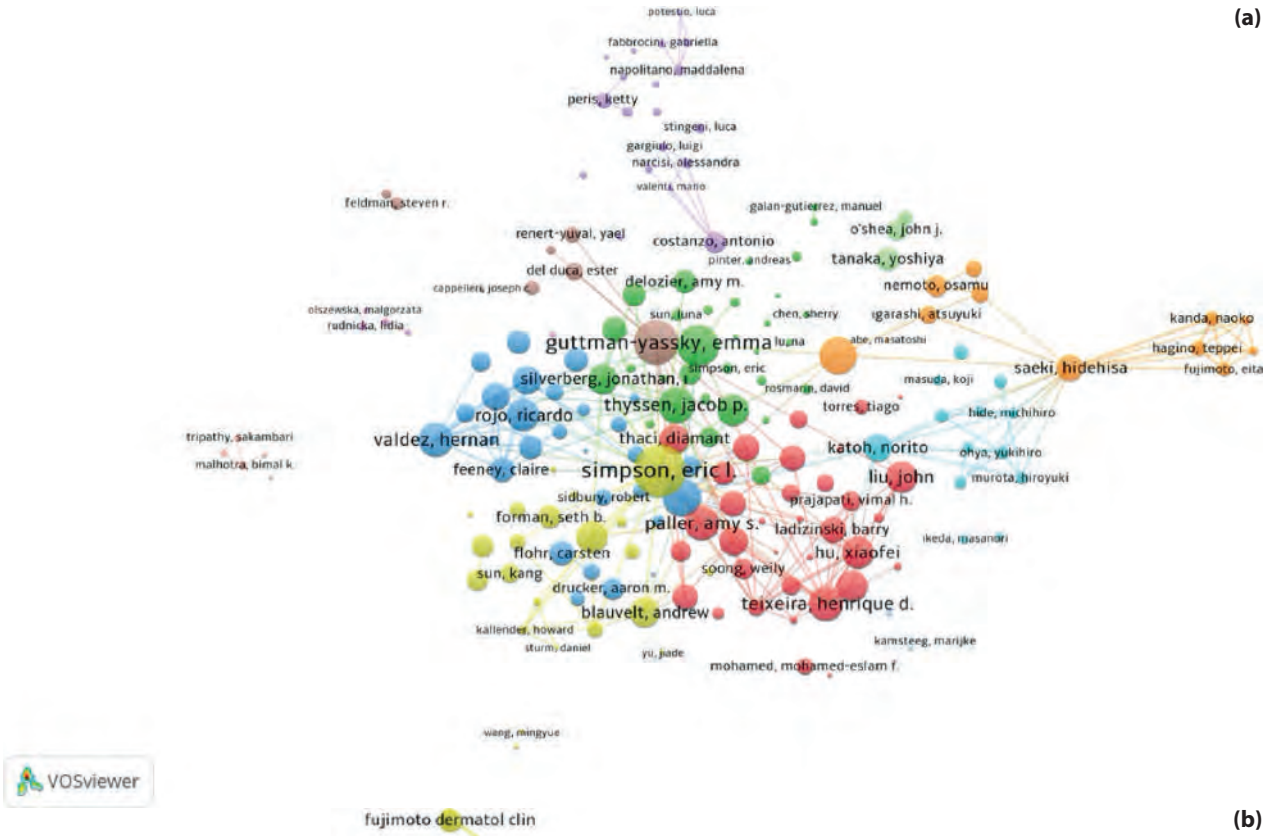
Figure 2a presents the author and institutional collaboration networks. The co-authorship network was constructed by including authors with five or more published papers and three or more citations. The analysis identified 12 clusters, 2,538 links,

Table 1. Academic Impact of authors based on PC, NP, PY start, h-index, g-index, and m-index

Author	h-index	g-index	m-index	TC	NP	PY start
Simpson EL	28	60	3.5	4894	60	2019
Guttman-Yassky E	26	43	2.364	3440	43	2016
Bieber T	22	36	2.2	4219	36	2017
Silverberg JI	22	55	2.75	3037	56	2019
Thyssen JP	20	44	2.857	2700	44	2020
Kabashima K	17	28	1.417	2638	28	2015
Silverberg J	17	27	2.125	1236	27	2019
Paller AS	16	31	1.6	2261	31	2017
Saeki H	16	25	3.2	647	33	2022
Valdez H	16	26	2.286	2007	26	2020

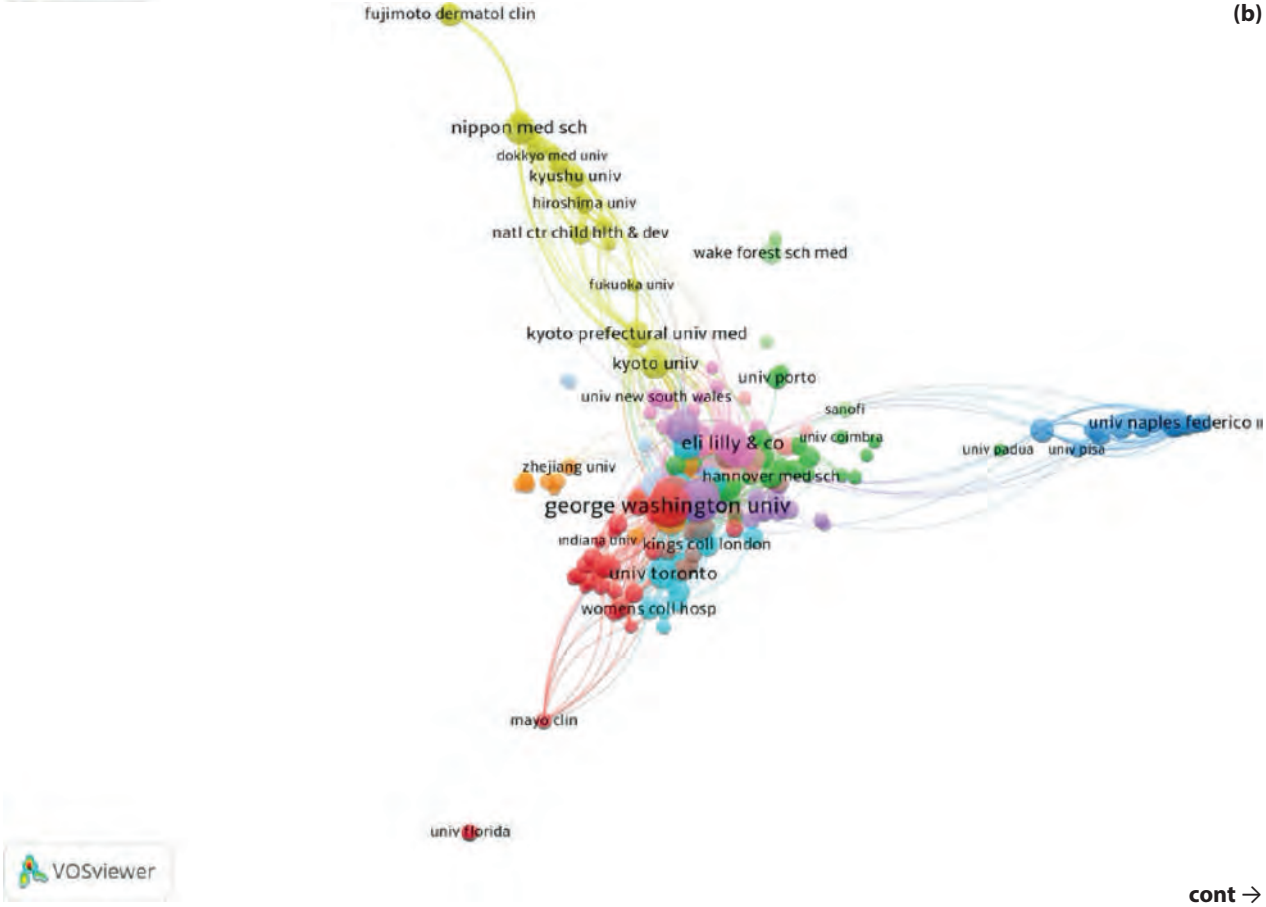
NP: Number of publications; PC: Publication count, defined as the total number of publications within the analysis period; PY start: Year of first publication; h-index: The maximum number h such that the author has published h papers, each cited at least h times; g-index: The largest number g such that the top g articles received at least g^2 citations in total; m-index: h-index divided by the number of years since the first publication.

(a)



VOSviewer

(b)



VOSviewer

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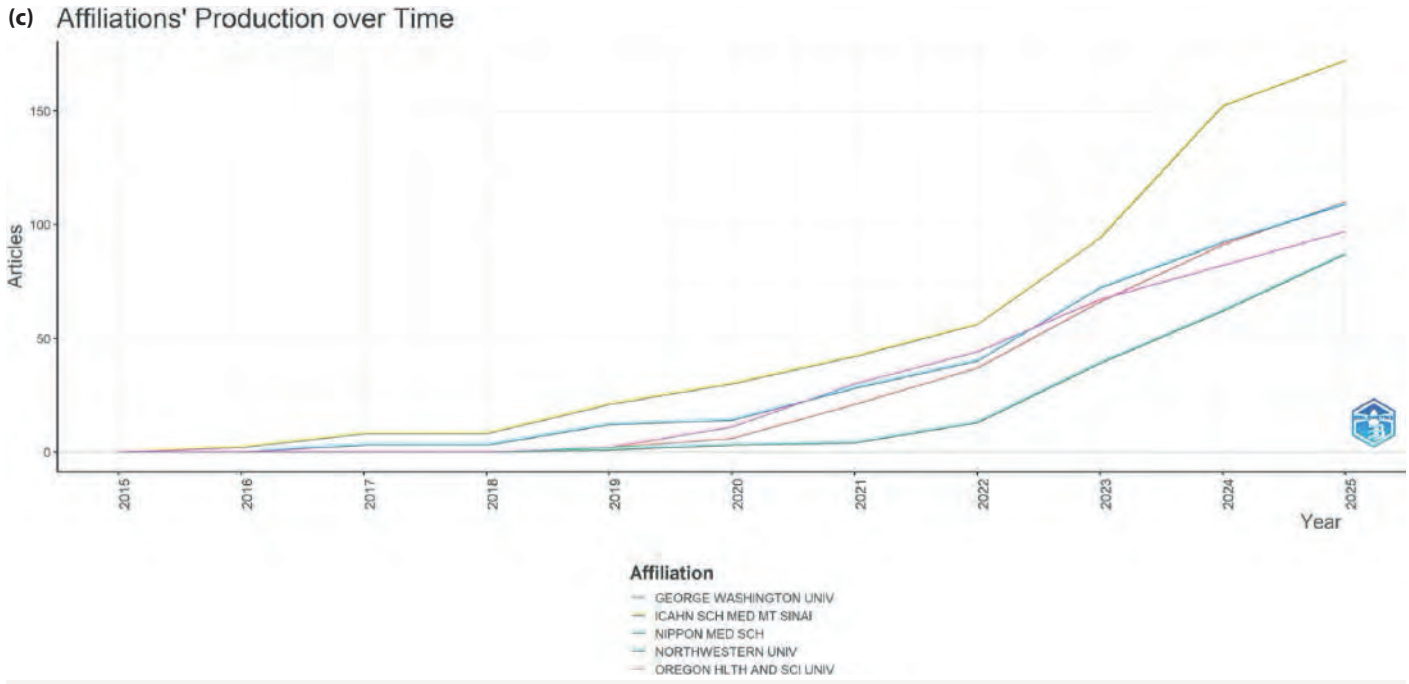


Figure 2. Connections between authors and institutions. **(a)** Co-authorship network analysis performed using VOSviewer; **(b)** co-institution network analysis performed using VOSviewer; **(c)** Affiliations' productivity over time analyzed using Biblioshiny.

and 184 authors. Within this network, Simpson EL emerged as the most influential author, contributing to four clusters, with 108 links and 4,894 citations, an h-index of 28, and a g-index of 60 (Table 1). Guttman-Yassky was ranked second in influence and was associated with eight clusters, 74 links, and 3,440 citations, with an h-index of 26 and a g-index of 43 (Table 1). The co-organization network analysis, which included organizations with five or more published papers and three or more citations (Fig. 2b), identified 12 clusters, 3,056 links, and 222 organizations. Oregon Health and Science University emerged as one of the most significant contributors to global collaborations and was associated with five clusters and 123 links. The Icahn School of Medicine had the highest number of publications, reaching approximately 175 by 2025 (Fig. 2c).

Country

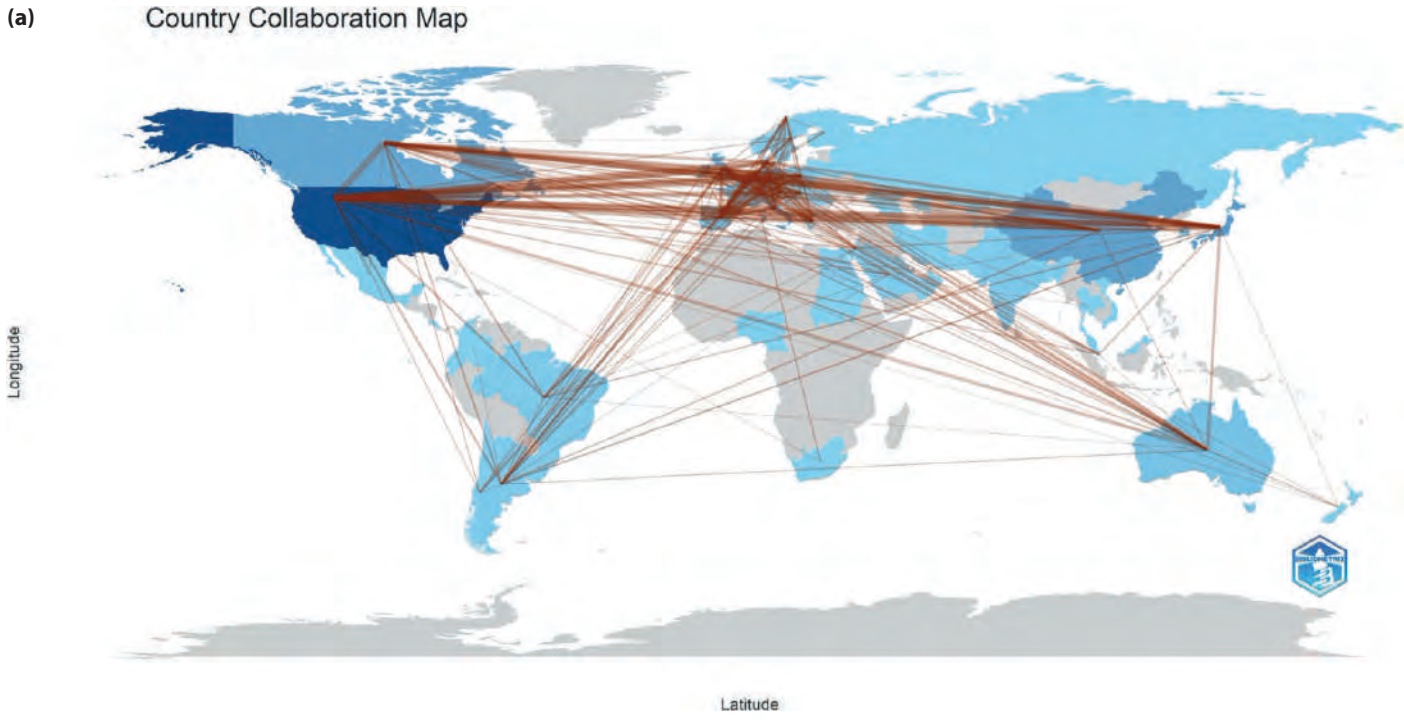
Collaborations among countries are illustrated on the world map (Fig. 3a). An inter-country collaboration network (Fig. 3b) was constructed by including countries with five or more published papers and three or more citations. The analysis identified six clusters, 410 links, and 41 countries. Within this network, the US emerged as the most collaborative country, contributing to all clusters, with 37 links and 518 documents. Germany was the second most collaborative country, with two clusters, 34 links, and 196 documents. The main corresponding authors' countries were the US, China, and Japan, respectively (Fig. 3c). The US produced the highest number of publications over the past decade, followed by Canada and Japan, demonstrating an upward trajectory with a marked increase in research output in recent years, reflecting growing interest in this field (Fig. 3d).

Keywords and Trend Topics

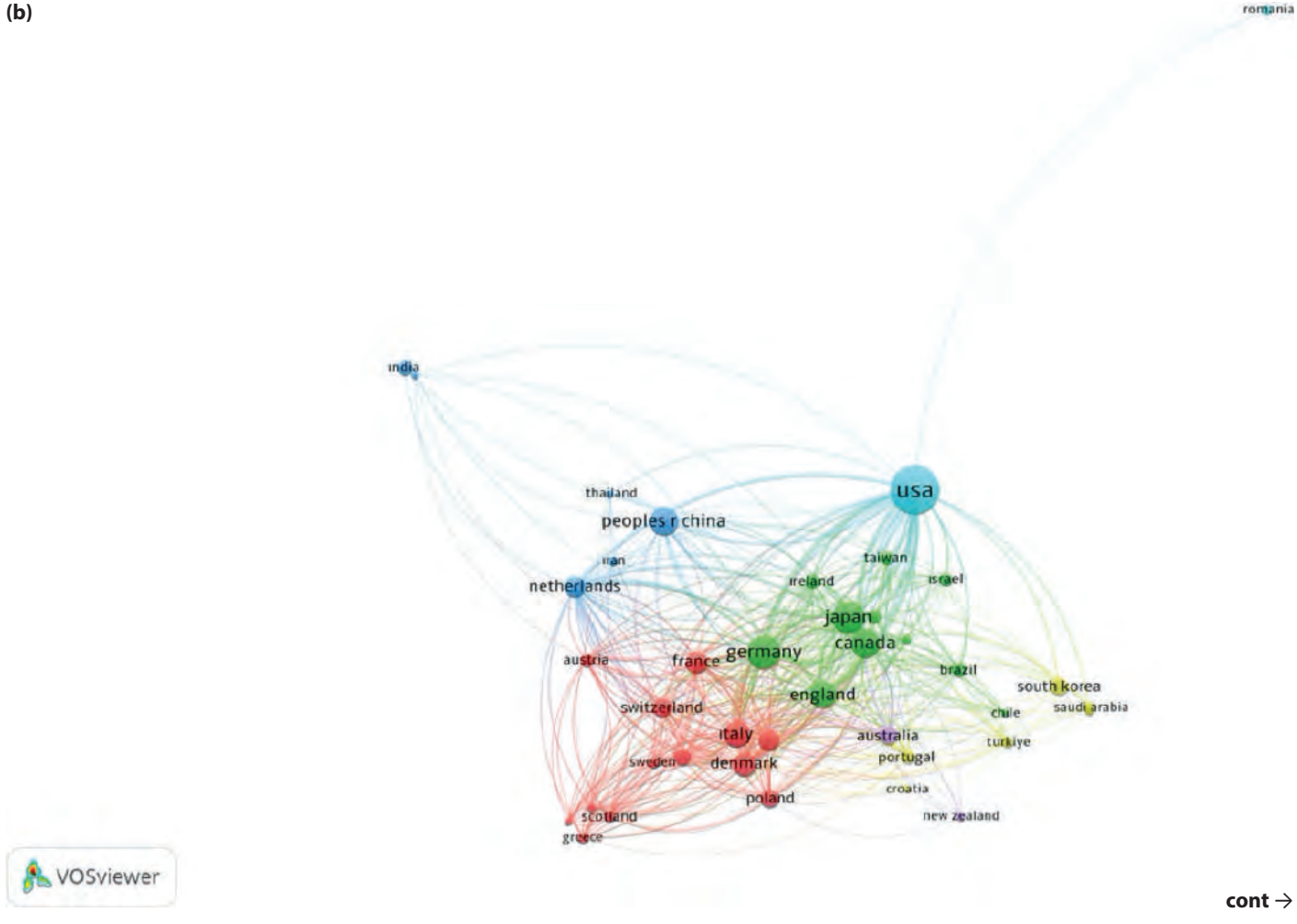
The keyword co-occurrence network analysis after synonym merging (Fig. 4a) generated 9 clusters with a total of 1,759 links, comprising 133 distinct keywords. Atopic dermatitis emerged as the most central and influential keyword, positioned at the core of the network with 129 co-occurrence links and 6 clusters. It demonstrated strong associations with JAK inhibitors, reflecting the pivotal role of these agents in current research trends. Among specific terms, upadacitinib, baricitinib, and eczema were also identified as highly frequent and strongly interconnected keywords, further highlighting the therapeutic and clinical relevance of JAK inhibition in AD research.

Keyword evolution analysis after removing irrelevant keywords and merging synonyms (Supplementary Material 1) revealed the frequency of occurrence of multiple key terms and their temporal trends (Fig. 4b). Research topics evolved from "hydrocortisone aceponate spray," which was the earliest focus, to "upadacitinib," "abrocitinib," and "phase 3 trials," which represent the latest focus. The analysis

(a)


Country Collaboration Map
Longitude
Latitude

(b)


romania
india
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france
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greece
VOSviewer

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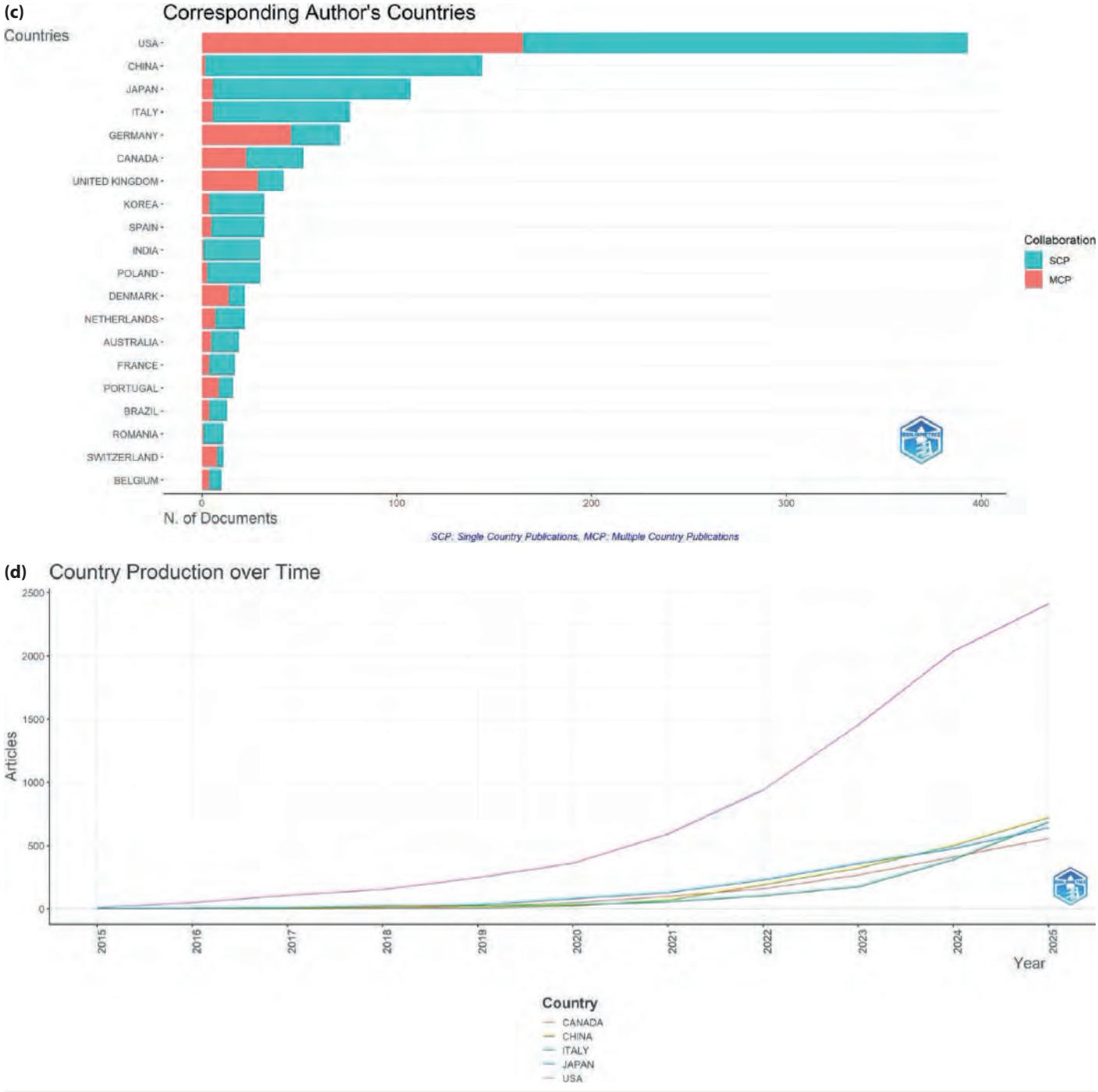
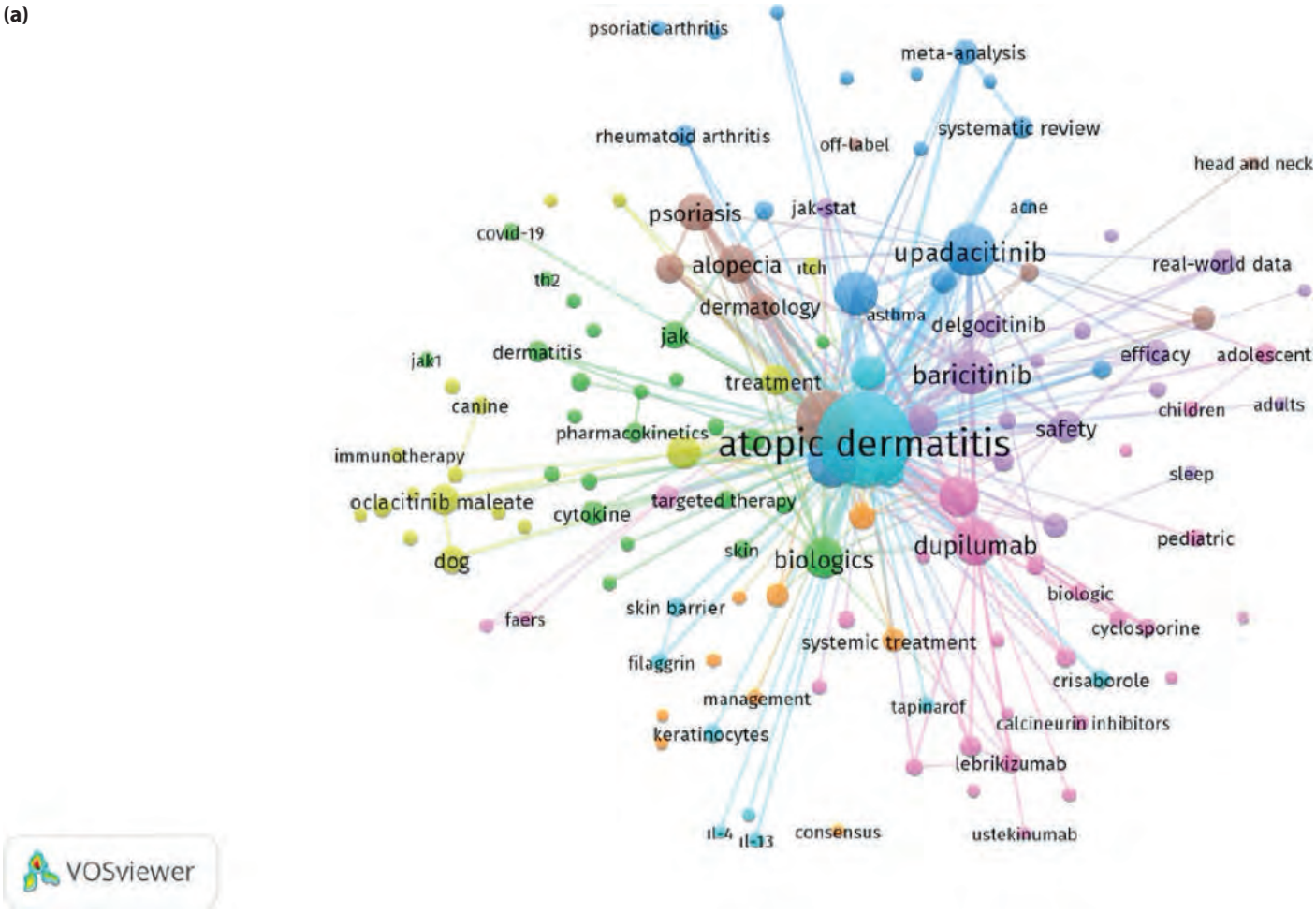


Figure 3. Country collaborations and research output. **(a)** Country collaborations shown on a world map and analyzed using Biblioshiny; **(b)** inter-country network analysis performed using VOSviewer; **(c)** corresponding authors' countries analyzed using Biblioshiny (pink: MCP, multiple-country publications; green: SCP, single-country publications); **(d)** countries' research output over time analyzed using Biblioshiny.

demonstrated that research on AD has shifted over time. Early studies predominantly focused on "filaggrin," "skin barrier," and "pruritus," reflecting an emphasis on disease pathophysiology. In contrast, more recent literature is characterized by the emergence of terms such as "JAK inhibitors," "atopic dermatitis," "double-blind," and "placebo,"

(a)


psoriatic arthritis
meta-analysis
rheumatoid arthritis
off-label
systematic review
head and neck
psoriasis
jak-stat
acne
covid-19
alopecia
itch
upadacitinib
real-world data
th2
dermatology
asthma
delgocitinib
jak
jak1
dermatitis
efficacy
adolescent
treatment
baricitinib
canine
children
adults
pharmacokinetics
atopic dermatitis
safety
immunotherapy
sleep
oclacitinib maleate
cytokine
targeted therapy
pediatric
dog
skin
biologics
dupilumab
biologic
skin barrier
faers
cyclosporine
systemic treatment
filaggrin
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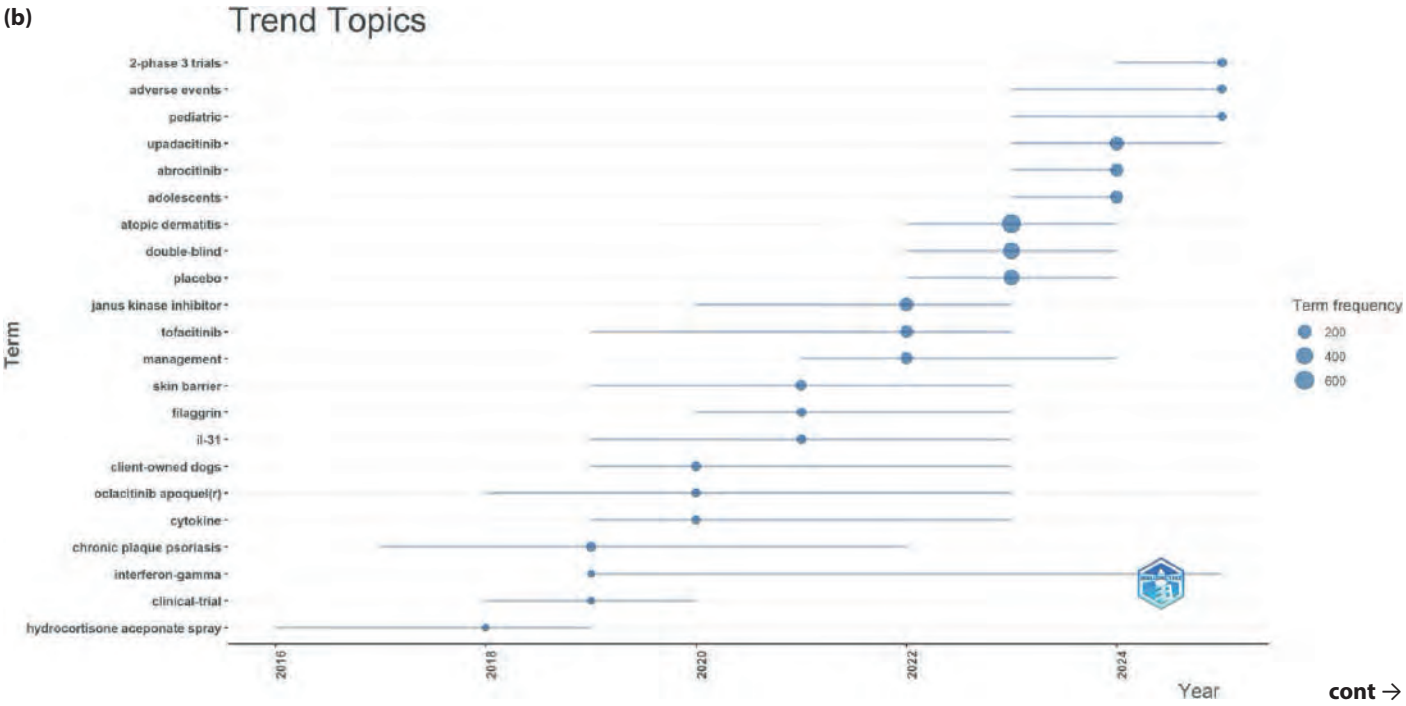
ustekinumab
VOSviewer

(b)


Trend Topics
2-phase 3 trials
adverse events
pediatric
upadacitinib
abrocitinib
adolescents
atopic dermatitis
double-blind
placebo
janus kinase inhibitor
tofacitinib
management
skin barrier
filaggrin
il-31
client-owned dogs
oclacitinib apoquel(r)
cytokine
chronic plaque psoriasis
interferon-gamma
clinical-trial
hydrocortisone aceponate spray
Term
2016
2018
2020
2022
2024
Year
Term frequency
200
400
600

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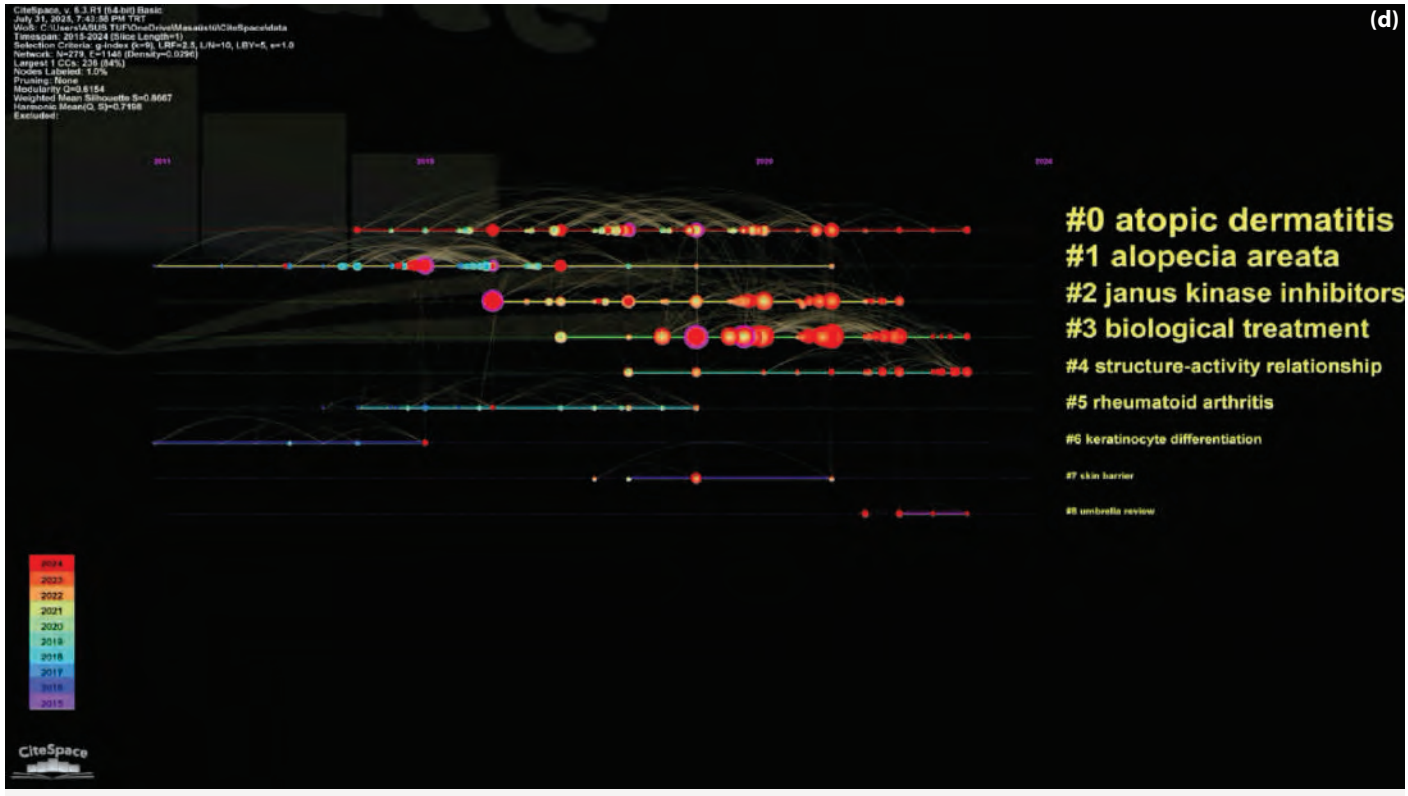


Figure 4. Keyword co-occurrence and evolution analyses. **(a)** Keyword co-occurrence analysis performed using VOSviewer; **(b)** Keyword evolution analysis performed using Biblioshiny; **(c)** Keyword co-occurrence clusters identified by CiteSpace in studies related to JAK inhibitors (2017–2024); **(d)** Temporal distribution of keyword clusters generated using CiteSpace.

Table 2. Most globally cited documents identified using biblioshiny

Paper	DOI	Total citations	TC per year	Normalized TC
Oetjen et al.[18]	10.1016/j.cell.2017.08.006	862	86.20	8.48
Xue et al.[19]	10.1038/s41392-023-01468-7	538	134.50	20.71
Bieber et al.[40]	10.1038/s41573-021-00266-6	491	98.20	13.16
Guttman-Yassky et al.[21]	10.1016/S0140-6736(21)00588-2	453	75.50	7.89
Blauvelt et al.[41]	10.1001/jamadermatol.2021.3023	425	70.83	7.40
Wollenberg et al.[42]	10.1111/jdv.14888	420	46.67	8.18
Simpson et al.[43]	10.1016/S0140-6736(20)30732-7	411	58.71	6.02
Tanaka et al.[44]	10.1038/s41584-021-00726-8	408	81.60	10.94
Damsky et al.[29]	10.1016/j.jaad.2016.12.005	396	39.60	3.90
Bieber et al.[45]	10.1056/NEJMoa2019380	379	63.17	6.60

TC per Year indicates the average number of citations received annually by each publication. Normalized TC represents citation counts adjusted for publication year to allow comparison between older and newer studies. DOI: Digital Object Identifier; NA: Not available; TC: Total citations.

indicating a transition toward clinical trials and therapeutic interventions, particularly randomized controlled studies. The keyword "adverse events" has appeared more frequently in recent years.

The cluster plot divided all keywords into nine categories. Pruritus and alopecia areata (AA) were the most frequently co-occurring keywords alongside AD, while delgocitinib and abrocitinib were the most commonly investigated JAK inhibitors (Fig. 4c). The timeline relationship of key clusters is shown in Figure 4d, with the current main focus being on "biological treatment" and "Janus kinase inhibitors."

References and Sources

The 10 most globally cited articles are shown in Table 2, indicating that, since 2015, research articles on AD published by Oetjen et al.[18] and Xue et al.[19] in Cell and Signal Transduction and Targeted Therapy, respectively, have been widely cited, with 862 and 538 global citations, respectively. Oetjen et al.[18] demonstrated that activation of the IL-4/IL-13 signaling axis and its downstream JAK1 pathway directly activates and sensitizes sensory neurons, playing a critical role in pruritus in patients with AD.[18] Xue et al.[19] demonstrated that JAK-STAT dysregulation, particularly JAK1 signaling, contributes to AD by promoting Th2 inflammation and pruritus.

The bibliographic coupling analysis of sources (Fig. 5a) included journals with at least three published papers and one or more citations. The co-cited journal network revealed 5 clusters connected by 4,970 links, comprising a total of 104 journals. Figure 5b displays the 20 documents with the highest burst citation intensity. Among them, "Bissonnette R, 2016" achieved the highest burst value (22.84) during 2017–2021. The article evaluated the efficacy and tolerability of topical tofacitinib in patients with mild-to-moderate AD, demonstrating significant improvements across endpoints with an early onset.[20]

The three-field plot (Fig. 5c) illustrates that landmark clinical trials, such as those by Guttman-Yassky et al.[21] in The Lancet in 2021 and Reich et al.[22] in The Lancet in 2021, were predominantly authored by Guttman-Yassky, Simpson, and Silverberg and focused on topics such as AD, dupilumab, and JAK inhibitors.[21,22] The dense connections reflect growing research interest in RCTs evaluating JAK inhibitors in comparison with placebo or dupilumab for the treatment of AD. The most frequently cited journals were The Lancet, The New England Journal of Medicine, The British Journal of Dermatology, and JAMA Dermatology, indicating that highly influential publications in this field predominantly appeared in high-impact clinical journals. Dermatology and Therapy published the highest number of studies in this field, with a marked increase observed after 2023. It was followed by the Journal of Dermatological Treatment (Fig. 5d).

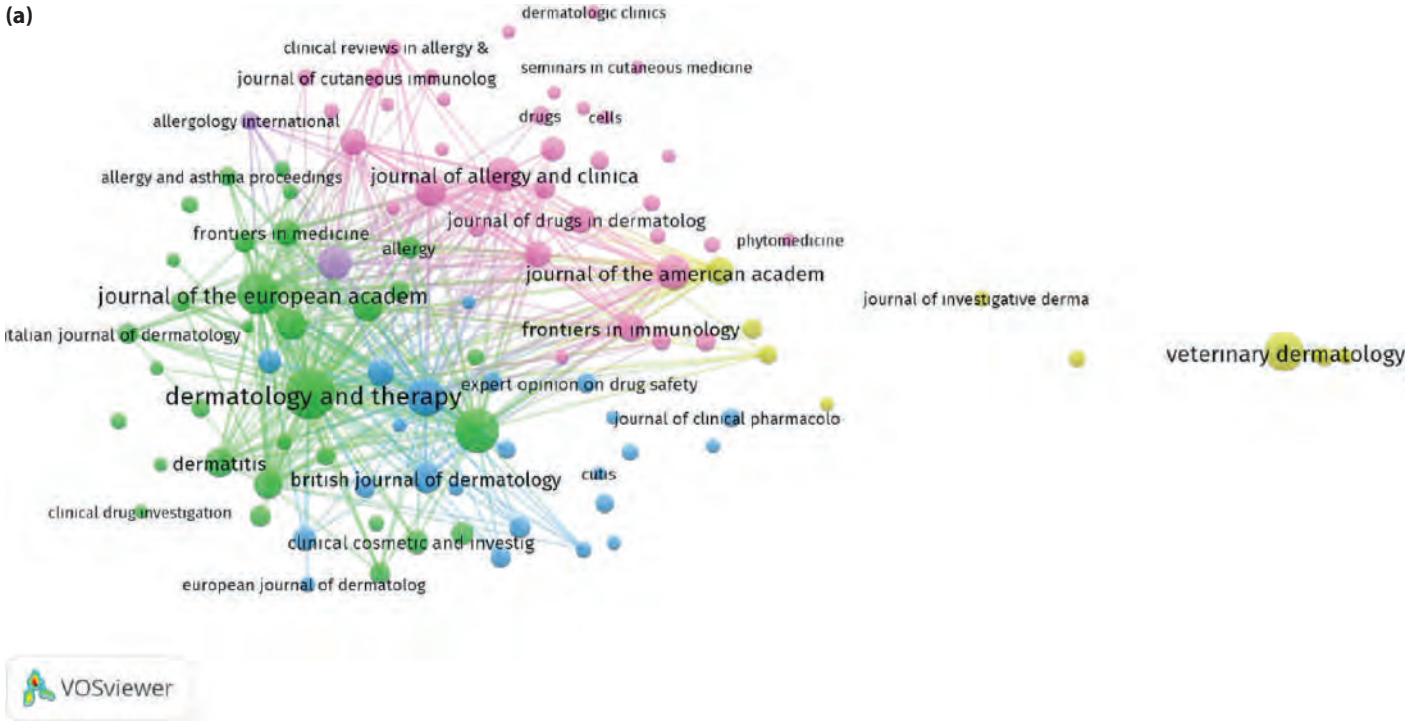
DISCUSSION

This bibliometric study is the first to comprehensively evaluate global research trends on JAK inhibitors in AD between 2015 and 2025. The findings indicate a marked increase in scientific output after 2020, closely associated with the growing clinical relevance and therapeutic use of JAK inhibitors in dermatology. Based on keyword, burst, and cluster analyses, the following major research themes were identified.

Pathophysiology and Skin Barrier Dysfunction

Early research activities centered on the fundamental mechanisms underlying AD, with an emphasis on epidermal barrier dysfunction, immune dysregulation, and pruritus. Keywords such as filaggrin, itch, and skin barrier dominated

(a)



(b)

Top 20 References with the Strongest Citation Bursts

References	Year	Strength	Begin	End	2015 - 2024
Bissonnette R, 2015, BRIT J DERMATOL, V172, P1395, DOI 10.1111/bjd.13551, DOI	2015	6.01	**2015**	2019	
Beck LA, 2014, NEW ENGL J MED, V371, P130, DOI 10.1056/NEJMoa1314768, DOI	2014	4.91	**2015**	2019	
Bao Lei, 2013, JAKSTAT, V2, Pe24137, DOI 10.4161/jkst.24137, DOI	2013	4.86	**2015**	2018	
Tanimoto A, 2015, INFLAMM RES, V64, P41, DOI 10.1007/s00011-014-0782-9, DOI	2015	4.6	**2015**	2020	
Levy LL, 2015, J AM ACAD DERMATOL, V73, P395, DOI 10.1016/j.jaad.2015.06.045, DOI	2015	15.87	**2016**	2020	
Bachelez H, 2015, LANCET, V386, P552, DOI 10.1016/S0140-6736(14)62113-9, DOI	2015	11.02	**2016**	2019	
Papp KA, 2016, BRIT J DERMATOL, V174, P1266, DOI 10.1111/bjd.14403, DOI	2016	6.98	**2016**	2019	
Papp KA, 2015, BRIT J DERMATOL, V173, P949, DOI 10.1111/bjd.14018, DOI	2015	4.94	**2016**	2019	
Genovese MC, 2016, NEW ENGL J MED, V374, P1243, DOI 10.1056/NEJMoa1507247, DOI	2016	2.77	**2016**	2020	
Bissonnette R, 2016, BRIT J DERMATOL, V175, P902, DOI 10.1111/bjd.14871, DOI	2016	22.84	**2017**	2021	
Amano W, 2015, J ALLERGY CLIN IMMUN, V136, P667, DOI 10.1016/j.jaci.2015.03.051, DOI	2015	11.83	**2017**	2020	
Simpson EL, 2016, NEW ENGL J MED, V375, P2335, DOI 10.1056/NEJMoa1610020, DOI	2016	10.48	**2017**	2021	
Nakagawa H, 2018, BRIT J DERMATOL, V178, P424, DOI 10.1111/bjd.16014, DOI	2018	8.88	**2018**	2021	
Guttman-Yassky E, 2018, J AM ACAD DERMATOL, V78, P872, DOI 10.1016/j.jaad.2018.01.016, DOI	2018	8.69	**2018**	2021	
Simpson EL, 2018, J AM ACAD DERMATOL, V78, P863, DOI 10.1016/j.jaad.2018.01.017, DOI	2018	7.75	**2018**	2021	
Blauvelt A, 2017, LANCET, V389, P2287, DOI 10.1016/S0140-6736(17)31191-1, DOI	2017	6.46	**2018**	2022	
Damsky W, 2017, J AM ACAD DERMATOL, V76, P736, DOI 10.1016/j.jaad.2016.12.005, DOI	2017	5.43	**2018**	2022	
Tanimoto A, 2018, EXP DERMATOL, V27, P22, DOI 10.1111/exd.13370, DOI	2018	4.69	**2018**	2021	
Cotter DG, 2018, J AM ACAD DERMATOL, V78, PS53, DOI 10.1016/j.jaad.2017.12.019, DOI	2018	2.63	**2018**	2021	
Guttman-Yassky E, 2019, J AM ACAD DERMATOL, V80, P913, DOI 10.1016/j.jaad.2018.01.018, DOI	2019	5.94	**2019**	2022	

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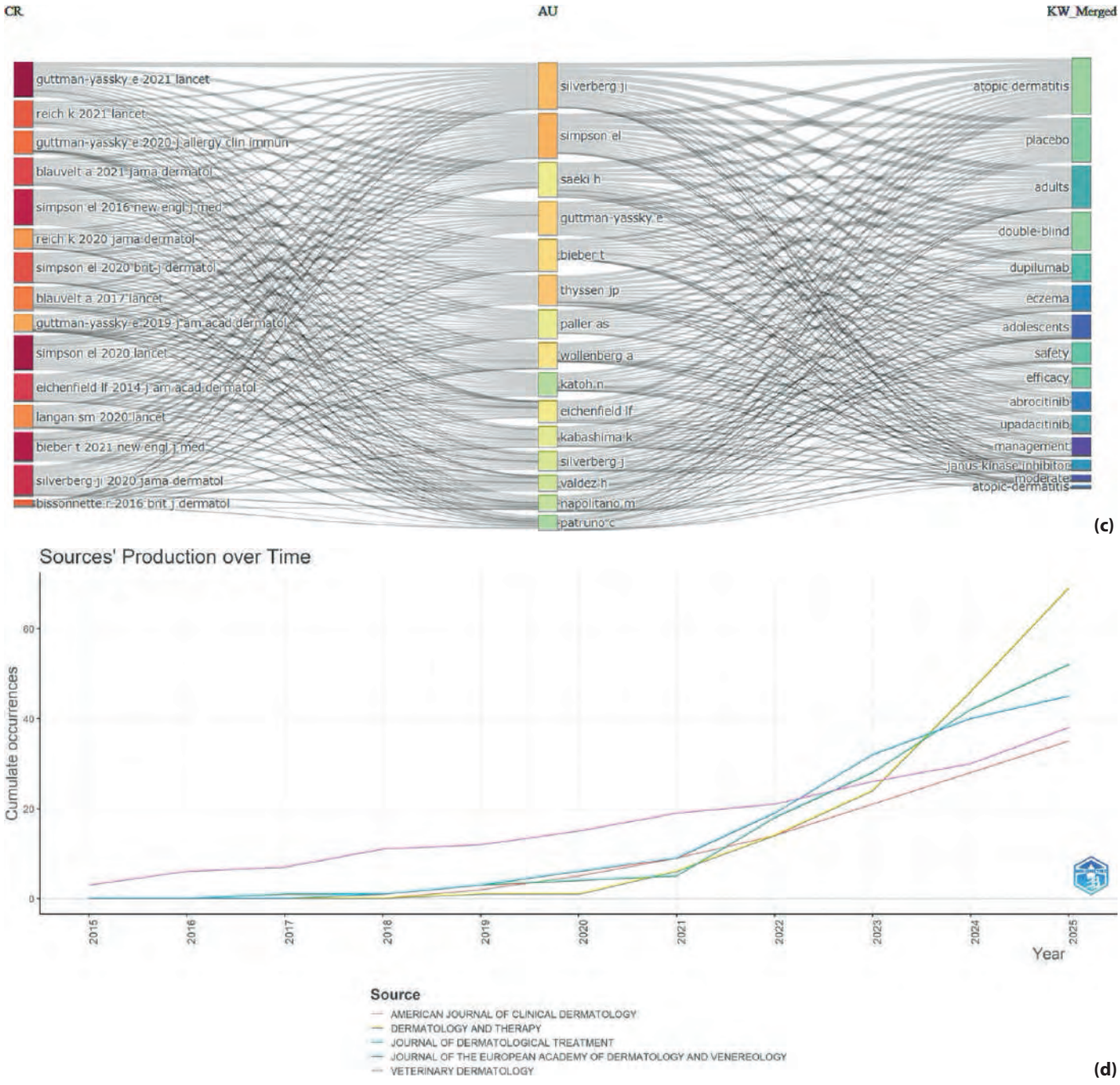

Figure 5. Source and citation analyses. **(a)** Co-cited journal network analysis performed using VOSviewer; **(b)** top 20 articles with citation bursts calculated using CiteSpace; **(c)** three-field plot illustrating relationships among the most cited references (CR), leading authors (AU), and frequently used keywords (KW), generated using Biblioshiny; **(d)** sources' production over time analyzed using Biblioshiny.

the literature between 2018 and 2024. The pathophysiology of AD is complex and has not yet been fully elucidated; however, it is critically influenced by three intersecting factors: dysregulation of the immune response, barrier dysfunction, and pruritus. Innate immune cells, including Th2 cells, type 2 innate lymphoid cells, and basophils, collectively produce key Th2 cytokines such as IL-4, IL-5, IL-13, and IL-31 in lesional skin.[23] Skin barrier dysfunction in AD results from filaggrin

mutations and cytokine-mediated suppression of barrier proteins. IL-4, IL-13, and IL-22 reduce filaggrin, involucrin, and loricrin expression, impairing epidermal integrity. Barrier injury triggers keratinocyte-derived IL-25, IL-33, and thymic stromal lymphopoietin (TSLP), activating Th2/Th17/Th22 and ILC2 responses that sustain inflammatory cytokine production and perpetuate chronic inflammation and barrier failure.[24,25] Chronic pruritus, defined as itch lasting more than 6 weeks, is a cardinal feature of AD, and research on this subject has increased markedly over the past decade. Scratching exacerbates barrier damage and contributes to the itch-scratch cycle. IL-31 directly induces pruritus, while IL-4 and IL-13 enhance neuronal activation and itch signaling.[26]

JAK Inhibitors

The therapeutic landscape of AD has evolved considerably over time, shifting from conventional corticosteroids to advanced biologic therapies, with particular emphasis on JAK inhibitors. The JAK-STAT pathway, a key mediator of cytokine signaling, involves JAK1, JAK2, JAK3, and TYK2, which activate STATs to regulate gene expression. Given the Th2-dominant nature of AD, JAK-STAT signaling is central to Th2 differentiation, providing a strong rationale for targeting this pathway with JAK inhibitors.[27]

Tofacitinib represents the first small molecule developed as a selective inhibitor of JAKs.[28] First-generation JAK inhibitors include tofacitinib, ruxolitinib, baricitinib, and oclacitinib, whereas second-generation inhibitors include upadacitinib, deucravacitinib, and abrocitinib.[29] Delgocitinib cream represents an emerging JAK inhibitor that has been increasingly highlighted in recent clinical studies for its efficacy in AD, particularly in patients with chronic hand eczema refractory to conventional topical corticosteroid therapy.[30,31] In recent years, ruxolitinib, particularly in its topical formulation, has emerged as a major focus of research and clinical interest in the management of AD. Clinical trials have demonstrated that topical ruxolitinib provides rapid and sustained relief of pruritus while exerting potent anti-inflammatory effects, thereby improving both objective disease severity scores and patient-reported outcomes. Importantly, long-term extension studies have confirmed its favorable safety profile, with low rates of application-site reactions and minimal systemic absorption, supporting its role as a safe and effective option for chronic use in patients with mild-to-moderate AD.[32] The emergence of topical agents such as ruxolitinib cream and delgocitinib reflects a growing trend toward safer and more localized treatment options.

Other Dermatologic Diseases

In the bibliometric analysis, AA emerged as one of the most frequently co-occurring keywords alongside AD. This finding reflects the increasing research overlap between these two immune-mediated skin diseases.[33] Epidemiological studies have demonstrated that individuals with AD are at higher risk of developing a broad spectrum of autoimmune comorbidities.[34] A comprehensive meta-analysis of 26 cohort studies, encompassing more than 1.6 million patients with AD and more than 15 million controls, demonstrated a significant association between AD and autoimmune diseases overall (HR=1.49; 95% CI=1.31–1.70), with statistically strong links to AA, celiac disease, rheumatoid arthritis, vitiligo, systemic lupus erythematosus, Sjogren disease, ulcerative colitis, and thyroid dysfunction.[35] Although AA is primarily mediated by Th1 pathways and AD by Th2 pathways, the two conditions are interrelated and share overlapping pathophysiologic mechanisms involving Th1, Th2, Th17, and Th22 imbalance. These converging mechanisms may partly explain their observed clinical coexistence and provide a rationale for investigating targeted immunomodulatory therapies that could potentially benefit both disorders. Beyond AD, JAK inhibitors have demonstrated efficacy in several other dermatologic disorders, highlighting their broad therapeutic potential. In psoriasis, both oral and topical JAK inhibitors have been shown to reduce disease severity by targeting cytokine signaling pathways central to disease pathogenesis, including IL-23, IL-17, and interferon signaling.[34] In both phase II and pivotal phase III trials, topical ruxolitinib 1.5% cream demonstrated significant efficacy in promoting repigmentation in patients with vitiligo.[36] These findings highlight the therapeutic versatility of JAK inhibition and indicate a paradigm shift toward targeted immune modulation.

Side Effects and Safety Profile

JAK inhibitors have demonstrated robust efficacy in the treatment of AD; however, their use is associated with an increased risk of specific infections and laboratory abnormalities. Although the potential risk of malignancy with JAK inhibitors has emerged as a growing concern, a recent network meta-analysis by Russell et al.[37] reported no increased risk of cancer compared with placebo or other agents, with a higher risk observed only in comparison with tumor necrosis factor inhibitors. Despite these findings, uncertainties regarding long-term safety persist and require cautious interpretation. Accordingly, appropriate patient selection, vigilant clinical and laboratory monitoring, and a shared decision-making approach are critical for optimizing therapeutic outcomes. Among adverse events, acne was identified as the most frequently reported side effect in the included studies.[38] This signal has been increasingly recognized in recent literature and may warrant further investigation as a potential class-related safety concern.

The use of JAK inhibitors in patients with AD has been associated with a modestly increased risk of thromboembolic events, including pulmonary embolism and deep vein thrombosis, compared with agents such as dupilumab and methotrexate.[39] Although the absolute risk remains relatively low, these observations highlight the importance of individualized patient selection, particularly in patients with pre-existing cardiovascular or thrombotic risk factors.

The broad immunomodulatory effects of JAK inhibitors require careful monitoring for potential side effects. Although JAK inhibitors are highly effective in the management of AD, clinicians must remain cautious regarding the potential for serious adverse events. The decision to initiate therapy should be individualized, with careful consideration of patient-specific risk factors. Before treatment initiation, appropriate screening for comorbidities and risk profiles is essential to ensure the safe and optimized use of these agents.

Limitations

This analysis was restricted to English-language publications and the WoSCC database, which may have resulted in the exclusion of relevant studies published in other languages or indexed in other databases, such as PubMed and Embase. Bibliometric analysis provides only quantitative data and does not assess the quality or methodological rigor of the studies. Moreover, citation-based metrics are time-dependent, favoring older articles and potentially underestimating the impact of newer research.

CONCLUSION

This bibliometric analysis provides a comprehensive overview of global research trends on JAK inhibitors in AD. The findings reveal a significant increase in scientific output and international collaboration. Although the efficacy of these agents has been well established, careful evaluation of their long-term safety and risk-benefit balance, as well as personalization of treatment, remains crucial. Overall, this study delineates the intellectual structure of research on JAK inhibitors, identifies emerging focus areas, and provides researchers with a roadmap for future investigations.

Ethics Committee Approval: Ethical approval is not required, as this study is an analysis of published literature.

Informed Consent: Written informed consent is not required, as this study is an analysis of published literature.

Conflict of Interest: The authors have no conflicts of interest to declare.

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Use of AI for Writing Assistance: The authors confirm that language model-based tools (specifically ChatGPT) were used solely for minor language editing and correction of grammar and syntax. The content, structure, and scientific interpretation of the manuscript were entirely developed and written by the authors. No AI tool was used for data analysis, interpretation, or drafting the manuscript.

Author Contributions: Concept – SA, SAT; Design – SAT, RD, AK; Supervision – SAT, RD, AK; Resource – SA, SAT; Materials – SA, SY; Data Collection and/or Processing – SA, SY; Analysis and/or Interpretation – SA, SY; Literature Review – SA, SY; Writing – SA, SY; Critical Review – RD, AK.

Peer-review: Externally peer-reviewed.

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Supplementary Material

For the statistical analysis of keyword trends using Biblioshiny, we removed irrelevant keywords and merged synonyms to improve the readability and accuracy of the results.

Removed: pde4 inhibitor, apremilast, ustekinumab

Synonyms:

clinical trial, trial
clinical trial, controlled-trial
chronic plaque psoriasis, to-severe psoriasis
skin barrier, barrier
tofacitinib, tofacitinib citrate

In VOSviewer, during the co-occurrence analysis of keywords, we merged synonyms to improve the consistency and accuracy of the results.

label	replaced by
adolescents	adolescent
adverse effects	adverse event
adverse events	adverse event
alopecia areata	alopecia
alopecia universalis	alopecia
atopic	atopic dermatitis
atopic eczema	atopic dermatitis
biological therapy	biologics
clinical trials	clinical trial
cytokines	cytokine
dogs	dog
eczema area and severity index	EASI
eczema area and severity ındex	EASI
effectiveness	efficacy
jak inhibitor	jak-inhibitor
jak inhibitors	jak-inhibitor
jakinibs	jak-inhibitor
keratinocyte	keratinocytes
janus kinase	janus kinases
janus kinase inhibitor	janus kinase inhibitors
therapeutics	therapy
targeted therapies	targeted therapy
systemic therapy	systemic treatment
oclacitinib	oclacitinib maleate
real-world	real-world data
real-world evidence	real-world data
stat	stat pathway

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Tumor Lysate-Stimulated Dendritic Cell Production: A Cross-Sectional Study

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ABSTRACT

Objective: Despite standard treatments (surgery, chemotherapy, and radiotherapy), resistance or recurrence may still develop in malignant diseases. Therefore, immunotherapeutic approaches, particularly dendritic cell–based tumor vaccines, are gaining importance as emerging treatment strategies. This study investigated the feasibility of using blood storage bags as culture systems for dendritic cell production in tumor vaccine preparation as an alternative to expensive Good Manufacturing Practice laboratory conditions. Furthermore, dendritic cell stimulation was evaluated using allogeneic tumor lysate.

Materials and Methods: CD34+ stem cells and mononuclear cells collected from donors via apheresis were combined with allogeneic tumor lysate in blood storage bags. Mature dendritic cell differentiation was induced by adding cytokines and prototypical immunoregulatory cytokines that stimulate bone marrow cell proliferation and support mature leukocyte formation.

Results: Flow cytometric analyses revealed that the targeted levels of mature dendritic cells were not achieved in cultures using blood storage bags. However, subsequent experiments demonstrated that mature dendritic cells could be generated from allogeneic tumor lysate under appropriate culture conditions.

Conclusion: These results suggest that economical and practical culture systems may be developed; however, standardization of culture conditions remains critical.

Keywords: Blood storage bag, culture medium, dendritic cell, immunotherapy, tumor vaccine.

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INTRODUCTION

Surgery, chemotherapy, and radiotherapy have traditionally been the primary approaches for treating malignant diseases. Although these methods provide positive responses in many patients, treatment resistance, relapse after remission, and inadequate responses, particularly in advanced tumors, remain significant clinical challenges. Resistance to chemotherapy and radiotherapy is associated with multifaceted mechanisms, including genetic and epigenetic adaptations of

tumor cells, immunosuppressive characteristics of the tumor microenvironment, and reprogramming of cellular signaling pathways. These factors limit the efficacy of current standard treatment protocols when used alone and necessitate the development of new strategies involving more effective, targeted, immunomodulatory, or combination therapies.[1–3] Current knowledge indicates that the dynamic interaction between the tumor microenvironment and the immune system plays a decisive role in cancer development and progression. Consequently, cell-based immunotherapies have emerged as an important treatment option.[2,4] The primary goal of this treatment approach is to activate immune system cells involved in generating tumor-specific responses, enhance their functions, and increase their capacity to recognize and eliminate tumor cells.[5] Thus, this approach aims to selectively identify and eliminate tumor cells by redirecting the organism's own immune response. Cell-based immunotherapies are becoming increasingly important in cancer treatment because they offer a more flexible and targeted approach to addressing tumor heterogeneity and treatment resistance compared with conventional therapies.[5,6]

Major histocompatibility complex (MHC) molecules play a critical role in initiating this immune response. MHC class molecules process antigenic structures and present them to T cells, thereby enabling cellular immune responses.[7] However, T-cell activation requires not only antigen recognition but also the appropriate presentation of that antigen by professional antigen-presenting cells (APCs).[8] Dendritic cells (DCs) are professional antigen-presenting cells with the highest capacity for antigen processing and presentation and are considered an effective cellular vaccine platform in cancer immunotherapy.[9–11]

For dendritic cell vaccines to be implemented in clinical practice, the manufacturing process must comply with Good Manufacturing Practice (GMP) standards. This includes the preparation of tumor-derived antigens (e.g., tumor lysate) under sterile conditions and the in vitro differentiation, maturation, and antigen loading of dendritic cells from peripheral blood-derived mononuclear cells, all under controlled cell culture protocols.[10,11] The resulting dendritic cells must meet clinical criteria for purity, viability, and functional activity before patient administration.[9] However, this production process requires costly GMP-compliant infrastructure, sterile cell culture equipment such as laminar flow cabinets and CO_2 incubators, and automated cell processing systems to standardize cell isolation and maturation steps. Therefore, dendritic cell vaccine production cannot be routinely implemented in many centers due to factors such as cost, the need for specialized personnel, and limited laboratory infrastructure.[12,13] This study aims to demonstrate that dendritic cell production can be achieved cost-effectively, even without access to specialized laboratory facilities. This approach may increase the accessibility of immune-based tumor vaccines and expand opportunities for future clinical applications and research. Such cost-effective experimental platforms may also facilitate preliminary research in resource-limited settings before transitioning to fully GMP-compliant manufacturing systems.

KEY MESSAGES

- Standard dendritic cell culture conditions are essential for achieving clinically relevant dendritic cell maturation, even when simplified and cost-effective culture systems are used.
- The successful generation of mature dendritic cells using allogeneic tumor lysates supports the translational potential of this approach for dendritic cell–based cancer immunotherapy.
- Optimization of low-cost culture strategies may facilitate broader clinical access to dendritic cell vaccines, particularly in centers with limited GMP infrastructure.

MATERIALS AND METHODS

Study Place and Design

This observational study included adult patients (aged ≥18 years) with malignant hematological disorders who were followed in the Division of Hematology, Department of Internal Medicine, Erciyes University Faculty of Medicine, between [08/2014] and [08/2015]. Age- and sex-matched healthy donors referred to the Apheresis Unit of Erciyes University were also included as controls.

Ethical Approval

This study was approved by the Erciyes University Clinical Research Ethics Committee (Approval Number: 2014/92; Date: 07.02.2014). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and Data Collection

Of the five patients included in the study, three were diagnosed with acute myeloid leukemia (AML), one with B-cell acute lymphoblastic leukemia (B-ALL), and one with mantle cell lymphoma (MCL). The patients' ages ranged from 18 to 64 years; four were female and one was male. The donor group consisted of five healthy volunteers, including two females and three males, with ages ranging from 18 to 62 years. Four donors were allogeneic donors, and one was an autologous stem cell transplant donor (Table 1).

Table 1. Patient and donor characteristics

Variable	Patient group (n=5)	Donor group (n=5)
Age (min–max)	18–64	18–62
Sex (F/M)	4/1	2/3
Clinical diagnosis	AML (n=3), B-ALL (n=1), MCL (n=1)	—
Cell collection method	—	Apheresis (peripheral stem cell collection)
$CD34^+$ cell dose ($\times 10^6$/kg)	—	1.0–1.7
Cell viability rate (%)	—	≥99.5

F: Female; M: Male; AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MCL: Mantle cell lymphoma.

Inclusion Criteria

Participants were eligible for inclusion if they were aged ≥18 years; had a morphologically and immunophenotypically confirmed diagnosis of hematologic malignancy (B-ALL, AML, or MCL) in the relapsed or refractory stage for tumor lysate preparation; or were healthy volunteer donors (related or unrelated) eligible for granulocyte colony-stimulating factor (G-CSF) mobilization and apheresis. Donors were required to have complete apheresis records, adequate post-collection cell viability (≥90%), and sufficient $CD34^+$ and mononuclear cell (MNC) counts. For all samples, comprehensive laboratory data, including flow cytometric evaluation of dendritic cell maturation markers (CD83, CD86, HLA-DR, and CD14), were required.

Exclusion Criteria

Patients and donors were excluded if they had evidence of active viral (HBsAg, anti-HCV, anti-HIV), bacterial, or fungal infection; documented autoimmune or chronic inflammatory disease; or pregnancy or lactation. Donors were additionally excluded if they had received immunosuppressive therapy or cytotoxic agents within four weeks prior to mobilization. Samples were excluded from the final analysis if microbial contamination occurred during incubation, post-apheresis cell viability was <90%, or flow cytometric data for dendritic cell maturation markers were incomplete. Only individuals and samples meeting all eligibility criteria were included in the final analysis.

Clinical, Surgical, and Laboratory Investigations

All clinical and laboratory data were collected prospectively using standardized data collection forms. Demographic characteristics, disease subtype, relapse status, donor type, and apheresis-related parameters were systematically recorded. Laboratory variables included $CD34^+$ cell counts, mononuclear cell yields, cell viability, and flow cytometric markers used to assess dendritic cell maturation. All data were anonymized and processed in accordance with institutional and ethical standards.

Peripheral blood samples were collected from patients to prepare tumor cell lysates, which were subsequently used as antigen sources for dendritic cell maturation. G-CSF (1 MIU/kg/day) was administered for 5 days to mobilize CD34(+) stem cells and mononuclear cells into the peripheral circulation of donors. On the sixth day, CD34(+) stem cells and mononuclear cells were collected via apheresis at a target dose of 1×10^6/kg.

The resulting CD34(+) cell and mononuclear cell suspension was combined with the patient-derived tumor lysate in a blood storage bag under sterile conditions. Granulocyte-macrophage colony-stimulating factor (GM-CSF) (800 U/mL) and interleukin 4 (IL-4) (1000 U/mL) were added to the culture medium to initiate dendritic cell differentiation. The cells were incubated, and samples were collected at 48–72 and 96–120 hours of culture to assess CD83, CD86, CD14, and HLA-DR expression by flow cytometry.

A total of five separate treatments were performed, with each patient's lysate paired with a single donor cell sample. Throughout the culture process, culture conditions (incubation temperature, CO_2 supplementation, and culture medium type) were progressively optimized to enhance cell maturation.

Obtaining Tumor Cell Lysate from Patients

Two 2-mL peripheral blood samples were collected intravenously from patients enrolled in the study during the relapse period using EDTA tubes. The first sample was analyzed by flow cytometry for disease-specific immunophenotypic assessment. After immunophenotyping, the second sample was stored at +4°C for a maximum of 24 hours for use as a tumor cell lysate.

Mechanical or chemical lysis procedures, which are necessary to obtain cells from tumor tissue in autologous tumor vaccine applications, were not applied in this study because malignant cells circulating in peripheral blood are already suitable for lysis. Therefore, no additional processing or culture manipulation was required for blood samples used as tumor cell lysates; the samples were prepared only for direct use in dendritic cell stimulation after short-term controlled cold storage.[14]

Peripheral blood samples containing circulating malignant cells were used directly as tumor antigen sources without additional mechanical or chemical lysis procedures.

CD34(+) Stem Cell and Mononuclear Cell Collection from a Healthy Donor

Healthy donors participating in the study received subcutaneous G-CSF (1 MIU/kg/day) administration for 5 days according to the standard mobilization protocols of the Apheresis Unit at Erciyes University. On the fifth day, complete blood count and peripheral blood CD34(+) cell levels were assessed. Apheresis was initiated when the peripheral blood CD34(+) cell count reached ≥20 cells/µL. Apheresis was performed using the Fresenius apheresis system for allogeneic donors and the Amicus apheresis system for autologous donors. CD34(+) stem cells and mononuclear cells were collected from each donor at a target dose of 1×10^6 CD34(+) cells/kg. The presence and viability of CD34(+) cells in the collected samples were confirmed by flow cytometry according to the Erciyes University Transplant Center CD34(+) Cell Analysis Standard Operating Procedure (SOP No: 196).[15]

Incubation Process Using Blood Storage Bags

To provide a low-cost, closed-system incubation environment for dendritic cell culture, 450 mL polyvinyl chloride (PVC) blood storage bags containing CPDA-1, CPD, or SAG-M anticoagulants were used in this study. All procedures were performed under sterile conditions in a laminar flow cabinet. A suspension containing 1×10^6/kg CD34(+) stem cells and mononuclear cells obtained from donors via apheresis was transferred into the bag according to the measured cell volume. In this study, dendritic cells were generated from donor-derived peripheral blood mononuclear cells containing mobilized CD34(+) hematopoietic progenitor cells collected by apheresis following G-CSF stimulation. Subsequently, 2 mL of tumor cell lysate stored at +4°C was added to the bag.

Before incubation, a 0-hour sample was collected, and CD34(+), CD14, HLA-DR, CD83, and CD86 expression levels were assessed by flow cytometry to establish a baseline profile. GM-CSF (800 U/mL) and IL-4 (1000 U/mL) were then added to induce the differentiation of PBMCs into immature dendritic cells. The bags were incubated under the conditions determined during the optimization phase of the study. During this phase, incubation was ultimately performed at 37°C in a humidified incubator with 5% CO_2, which was identified as the optimal condition for dendritic cell differentiation. According to the literature, dendritic cell maturation progresses from an immature to a mature phenotype at 48–72 hours and 96–120 hours, respectively.[16] Therefore, serial samples were collected from the bags at 48 or 72 hours and 96 or 120 hours, and the maturation process was monitored by flow cytometry through increased CD83 and CD86 expression and decreased CD14 expression.

Flow Cytometric Analysis of Tumor Cell-Stimulated Mononuclear Cells

At the specified incubation time points, 2 mL of samples were collected from the blood storage bag and transferred into EDTA tubes. A 100 µL aliquot of each sample was transferred to a TruCount® tube, and 20 µL of fluorescently labeled antibodies against the surface markers CD14, CD34, CD45, HLA-DR, CD83, CD86, and CD209 were added. After vortex mixing, 500 µL of OptiLyse lysing solution was added to the tubes, and the samples were incubated in the dark for 10 minutes. The cell suspensions were then washed with 2 mL of isotonic solution by centrifugation at 1500 rpm for 5 minutes, and this procedure was repeated three times. The resulting cell pellets were analyzed by flow cytometry. Flow cytometric analyses were performed using a multicolor flow cytometer (Navios™, Beckman Coulter, Brea, CA, USA), and at least 10,000 events were acquired and analyzed for each sample.

The dendritic cell differentiation process was evaluated in three stages:

Hour 0 (baseline): The mononuclear cell phenotype was confirmed ($CD14^+/CD45^+/HLA\text{-}DR^+$), and maturation markers were negative as expected ($CD80^-$, $CD83^-$, $CD86^-$).

Hours 48–72 (immature DC phase): Immature dendritic cell development was assessed through $CD45^+/HLA\text{-}DR^+/CD86^+$ expression, along with a decrease in CD14 expression.

Hours 96–120 (mature DC phase): Maturation was confirmed by an increase in costimulatory markers $CD83^+$ or $CD80^+$ on $HLA\text{-}DR^+$ cells.

A dendritic cell population of ≥60% in flow cytometric analysis was used as the threshold for significant maturation.[17]

Optimization Studies

In this study, sequential optimization procedures were performed to determine the optimal culture microenvironment for DCs obtained from peripheral blood mononuclear cells (PBMCs) stimulated with tumor cell lysate. In each application, parameters associated with negative outcomes were systematically modified:

• Application 1: Lysate from a B-ALL patient + unrelated donor MNCs, with bag incubation at +4°C. Flow cytometric analysis at 48 and 96 hours showed that no mature DCs were detected.

• Application 2: Considering the variability in lysate immunogenicity among different disease groups reported in the literature, AML lysate was used instead. Because DC

Table 2. Flow cytometry findings by application

Application	Tumor lysate source	Incubation condition	CD83 (%)	CD86 (%)	CD14 (%)	Result
1	B-ALL	+4°C	0 → 0	35.6 → 27.6	Unchanged	No conversion
2	AML	+4°C (120 h)	0.01 → 0.09	37.2 → 34.3	↑31.6 → 35.4	No conversion
3A	AML	37°C bain-marie	No increase	No increase	—	No conversion
3B	AML	37°C + 5% CO_2	48 h: —; 120 h: ↑	55.8 → 81.6	↓	Conversion
4A	MCL	37°C bain-marie	0 → 0	0 → 0.06	—	No conversion
4B	MCL	37°C + 5% CO_2	34.4 → 74.8	61.4 → 76.9	↓	Conversion
5	AML (related donor)	37°C + 5% CO_2	4.4 → 15.3	32.2 → 25.6	Unchanged	Poor conversion

AML: Acute myeloid leukemia; B-ALL: B-cell acute lymphoblastic leukemia; MCL: Mantle cell lymphoma.

maturation may require 4–5 days and, in some cases, 6–7 days,[16] the incubation period was extended to 120 hours. Despite this modification, no maturation was observed.

• Application 3: The incubation microenvironment was optimized while maintaining incubation at +4°C.

3A: Incubation at 37°C in a bain-marie using the bag system without CO_2 supplementation.

3B: Incubation at 37°C with 5% CO_2 using standard culture dish conditions.

DC maturation was achieved in condition 3B, with a significant increase in CD83/CD86 expression, whereas condition 3A was insufficient.

• Application 4: The disease group was repeated using MCL lysate, while maintaining the 3A/3B setup. The strongest maturation response (CD83↑, CD86↑, CD14↓) was achieved under 37°C/5% CO_2 conditions; the bain-marie condition was again inadequate.

• Application 5: Unlike the previous applications, a related donor was used. Partial and low-grade maturation was observed under 37°C/5% CO_2 conditions.

In summary, +4°C incubation and CO_2-free bain-marie conditions did not support DC maturation. Incubation at 37°C with 5% CO_2 and transfer to a culture vessel were identified as critical determinants. Furthermore, the lysate source (AML vs. MCL) and donor relationship influenced the results.

Dendritic Cell Culture Protocol (Final and Applied Workflow)

1. Combination and Pre-Stimulation: Tumor cell lysate obtained from the patient was combined with MNCs collected from the donor in a blood storage bag under sterile conditions. DC differentiation was initiated by adding GM-CSF (800 U/mL) and IL-4 (1000 U/mL).
2. Transfer to Culture Medium: Approximately 20 mL of cell suspension was collected from the bag and transferred to Iscove's Modified Dulbecco's Medium (IMDM).
3. Pre-incubation: The suspension was incubated at 37°C with 5% CO_2 for 2–3 hours (adhesion phase).
4. Washing and Continued Incubation: Non-adherent cells were removed twice using PBS, and incubation of the remaining adherent cells was continued for 5 days under 37°C/5% CO_2 conditions.
5. Time Points and Analysis: Samples were collected at 48, 72, 96, and 120 hours, and the immature-to-mature DC transition was monitored by flow cytometry using CD14, HLA-DR, CD83, and CD86 markers.

Statistical Analysis

Due to the methodological nature of the study and the limited sample size, statistical hypothesis testing was not performed. Flow cytometric data were evaluated descriptively, and changes in expression levels over time were presented graphically.

RESULTS

The clinical diagnoses of the five patients included in the study were confirmed by flow cytometric analysis. AML was detected in three patients, B-ALL in one patient, and MCL in one patient. The viability rate of CD34(+) cells obtained by apheresis from the peripheral blood of five healthy donors was >99.5% in all samples (Table 1).

Dendritic cell maturation varied depending on the tumor cell lysate source, incubation temperature, and culture conditions. In the first two applications, which were incubated in blood bags at +4°C, no increase in CD83 or CD86 expression was observed, and mononuclear cells were not found to differentiate into the dendritic cell phenotype (Table 2).

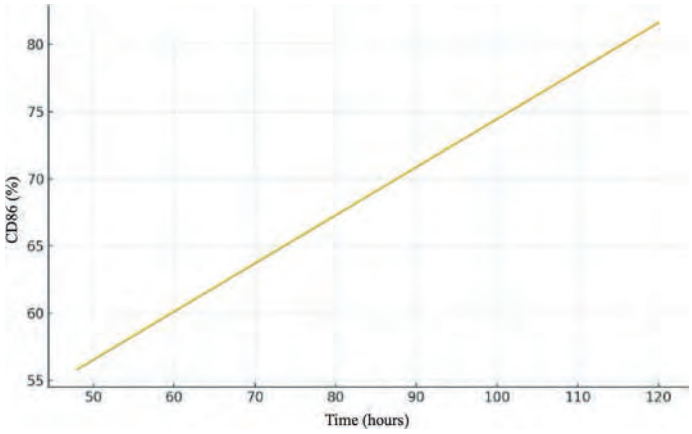

Figure 1. Application-3B: CD86 expression change.

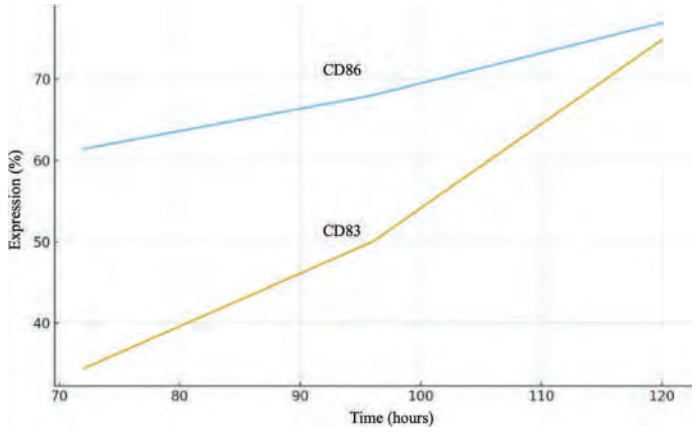

Figure 2. Application-4B: CD83 and CD86 expression change.

In the third application, some of the lysate-exposed cells were cultured in a 37°C bain-marie, whereas the remaining cells were cultured under 37°C and 5% CO_2 conditions. While no maturation was observed in the bain-marie arm, CD86 expression in the sample cultured in the incubator increased from 55.8% at 48 hours to 81.6% at 120 hours, indicating differentiation into dendritic cells (Table 2, Fig. 1).

In the fourth application, the lysate source was changed to mantle cell lymphoma. While no maturation occurred in the bain-marie environment, CD83 expression increased from 34.4% to 74.8% and CD86 expression increased from 61.4% to 76.9% between 72 and 120 hours under 37°C and 5% CO_2 conditions. A significant decrease in CD14 levels was also observed. This application demonstrated the most pronounced mature dendritic cell phenotype in the study (Table 2, Fig. 2).

In the fifth application, when cells from a related donor were used, a limited increase in dendritic cell markers was observed (CD83: 4.42% → 15.36%). The decrease in CD86 levels and the lack of a significant change in CD14 levels indicated that maturation was weaker and slower (Table 2).

Overall, the critical determinant of dendritic cell maturation was concluded to be culture conditions of 37°C and 5% CO_2. Additionally, the tumor type used for lysate preparation and the relative immune compatibility between the donor and patient may influence the magnitude of the response.

DISCUSSION

This study evaluated the use of a blood storage bag as a low-cost, closed platform for the production of DCs from tumor cell lysate-stimulated PBMCs. Different incubation microenvironments (temperature/CO_2), tumor lysate sources (B-ALL, AML, and MCL), and donor relationships (unrelated vs. related) were compared to assess their effects on DC maturation. The findings demonstrated that +4°C or 37°C bain-marie conditions without CO_2 supplementation did not support maturation, whereas the mature DC phenotype, defined by increased CD83/CD86 expression and decreased CD14 expression, was reliably achieved under culture conditions of 37°C and 5% CO_2. This pattern is consistent with classical literature emphasizing the strong dependence of DC biology on microenvironmental parameters, including pH, gas exchange, temperature, and cell-surface interactions.[10,18,19]

The Incubation Microenvironment Determines Maturation

DC maturation is a metabolically demanding process involving antigen uptake and processing, as well as increased expression of costimulatory molecules (particularly CD83 and CD86) and MHC-II (HLA-DR).[18,19] CO_2 supplementation and a temperature of 37°C are essential for the stable function of the bicarbonate buffer system; these conditions maintain cytokine signaling activity (GM-CSF/IL-4) and NF-κB-mediated maturation pathways.[10,18] In our study, the absence of maturation observed at +4°C and the failure of a CO_2-free bain-marie environment suggest that a microenvironment incompatible with these physicochemical requirements suppresses DC differentiation. Conversely, the reproducible confirmation of maturation through increased CD83/CD86 expression and decreased CD14 expression under 37°C and 5% CO_2 conditions reinforces the fundamental principles of standard monocyte-derived DC protocols.[12,20]

Blood Storage Bags are Practical in the Early Stages but Limited for Maturation

Although closed-bag systems offer advantages such as sterility and logistical convenience, particularly during initial assembly and pre-stimulation stages, their maturation capacity is limited. Restricted gas exchange within the bag, pH alterations, and insufficient surface adhesion may hinder DC maturation.

This study demonstrates that blood storage bags can be used for preparation, transport, and early stimulation; however, maturation requires a gas- and pH-controlled incubator and an appropriate culture surface. These findings are consistent with the widely accepted "preparation in bag → maturation in culture vessel" workflow used in clinical-scale DC production processes.[10,18,19]

Differences in Antigen Source and Immunogenicity are Important

Variations in maturation responses according to tumor lysate source (more pronounced with MCL lysate and weaker with B-ALL/AML lysates) may be associated with differences in the content of immunostimulatory components, such as DAMPs, neoantigens, nuclear and mitochondrial protein fragments, and heat shock proteins. Indeed, the success of the tumor lysate-loaded DC strategy depends not only on the quantity of antigen but also on the immunogenic characteristics of the cell death pattern underlying lysate formation.[19,21] Therefore, quantitative profiling of markers such as heat shock protein 70/90, high mobility group box 1, and calreticulin in lysates, as well as standardization of immunogenic cell death (ICD) protocols where possible, are recommended for future studies.

The limited increase in CD83 expression observed in the fifth application using cells obtained from a related donor may be consistent with reduced allostimulation due to HLA similarity and/or peripheral immune tolerance mechanisms. This finding suggests that, although DC vaccines primarily aim to achieve antigen specificity, in vitro maturation may partially benefit from allogeneic danger signals. When these signals are weak, the increase in costimulatory molecules may be limited.[10,18,21]

DC-based vaccines are important due to their potential to reduce the risk of relapse, particularly during periods of minimal residual disease or following curative treatments.[18–20] Our workflow combines a low-cost pretreatment phase with a standard 37°C and 5% CO_2 maturation phase, suggesting a feasible approach for centers with limited infrastructure. To demonstrate the clinical value of this approach, further validation is required through (i) functional assays (allo-MLR, IFN-γ ELISPOT, and IL-12p70 release), (ii) evaluation of antigen-specific T-cell activation, and (iii) in vivo safety and response assessments.[10,19,21]

While this study focuses on developing a practical and low-cost experimental approach for dendritic cell production, several factors must be addressed before clinical translation can be considered. In particular, biosafety and quality control considerations are essential for the potential clinical application of this strategy. In clinical-grade dendritic cell production, strict procedures, including sterility testing, endotoxin assessment, standardized antigen preparation, and validated functional assays to confirm antigen-presenting capacity, are required to ensure product safety and reproducibility. These processes are typically performed under GMP conditions using validated quality control assays.[22] Therefore, the present findings should be considered a preliminary proof-of-concept experimental framework that may guide future studies aimed at developing scalable, standardized, and clinically compliant dendritic cell production strategies.

The main limitations of this study include the small sample size and the heterogeneity of the patient population, which consisted of individuals with different hematological malignancies. These factors may limit the generalizability of the findings and should be interpreted with caution. In addition, the study did not include functional assays evaluating the antigen-presenting capacity of the generated dendritic cells, and the potential effects of the pouch material on cytokine or antigen adsorption were not investigated. Future studies should evaluate the production of ICD-standardized lysates and validate antigen/DAMP profiles using techniques such as mass spectrometry or multiplex ELISA. Furthermore, testing alternative culture systems, such as gas-permeable pouches or culture bags, may improve culture efficiency. Optimization of the multiplicity of infection and cell density may increase the consistency of achieving the maturation threshold (≥60%), and the controlled use of maturation enhancers, such as TLR agonists (e.g., poly-I:C or R848), may further enhance dendritic cell maturation.

CONCLUSION

The data suggest that the microenvironment (37°C, 5% CO_2, and surface interaction) is the primary limiting factor for DC maturation, whereas the use of blood bags, although practical during the early phase, is insufficient for maturation. These findings suggest that a low-cost yet biologically accurate production platform can be established and may be translated into clinical applications through functional validation and standardized antigen preparation.

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Multi-Omics Integration Identifies Ferroptosis-Related miRNA–mRNA Networks and Independent Prognostic miRNAs in Triple-Negative Breast Cancer

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ABSTRACT

Objective: This study aimed to comprehensively characterize the transcriptomic and proteomic landscape of ferroptosis-associated mRNAs, miRNAs, and proteins, elucidate subtype-specific regulatory networks, and identify potential prognostic biomarkers in triple-negative breast cancer (TNBC).

Materials and Methods: Using the TCGA-BRCA cohort for discovery and the METABRIC dataset for validation, we performed differential expression and functional enrichment analyses comparing TNBC and non-TNBC subtypes. An integrated miRNA–mRNA regulatory network was constructed, and multivariable Cox proportional hazards regression analysis was performed to evaluate the prognostic value of the identified biomarkers.

Results: TNBC exhibited profound transcriptional dysregulation, characterized by the marked upregulation of the iron-sequestering gene FTMT ($log_2FC=5.60$) and downregulation of the iron exporter SLC40A1 ($log_2FC=-3.13$). We identified 197 significantly dysregulated miRNAs, including the overexpressed oncomiR hsa-miR-135b and the suppressed tumor-suppressor miRNA hsa-miR-449a. Functional enrichment analyses consistently highlighted substantial disruptions in iron homeostasis and oxidative stress pathways. Network analysis identified NQO1 and SLC38A1 as central mRNA hubs. Notably, although specific mRNAs, including SLC40A1 and ALOX15, demonstrated prognostic value in univariate analyses, multivariable Cox regression analysis revealed that only hsa-miR-378c and hsa-miR-449a remained significant as independent prognostic factors.

Conclusion: This multi-omics integration maps the ferroptosis-related regulatory landscape of TNBC. Although specific mRNAs serve as key structural hubs, hsa-miR-378c and hsa-miR-449a emerge as robust independent prognostic biomarkers, providing important insights into TNBC risk stratification and potential targeted therapeutic strategies.

Keywords: Biomarker, breast cancer, ferroptosis, miRNA, transcriptomic analysis.

INTRODUCTION

Triple-negative breast cancer (TNBC) has received particular attention as an aggressive and treatment-resistant subtype of breast cancer.[1,2] Approximately 40% of patients with TNBC face a high risk of early recurrence and metastatic progression, contributing to poor clinical outcomes.[3] In recent years, ferroptosis, an iron-dependent form of regulated cell death distinct from apoptosis and necrosis, has emerged as a promising therapeutic target in cancer biology because of its potential to eliminate treatment-resistant malignancies by exploiting the metabolic vulnerabilities of cancer cells.[4–7]

MicroRNAs (miRNAs) play a pivotal role in post-transcriptional gene regulation and profoundly affect cancer initiation, progression, and therapeutic response.[8,9] By targeting ferroptosis-related genes, miRNAs can critically modulate ferroptotic processes, underscoring their potential value as biomarkers and therapeutic targets.[10,11] Furthermore, elucidating miRNA-regulated ferroptosis pathways may provide new insights into the molecular pathogenesis of TNBC and facilitate the development of personalized treatment strategies.

However, despite the growing recognition of the role of ferroptosis in cancer, the current literature provides limited data on subtype-specific expression patterns and the prognostic value of ferroptosis-related genes and their regulatory miRNAs in breast cancer. Comprehensive molecular analyses of ferroptosis pathways are therefore essential, particularly in TNBC, to identify novel biomarkers and therapeutic targets. In addition, mapping the interaction networks between ferroptosis-related genes and their regulatory miRNAs is critical for understanding disease mechanisms and developing targeted therapeutic approaches. To address this gap, this study aimed to characterize ferroptosis-related mRNA and miRNA expression patterns in TNBC, identify key biological pathways associated with iron homeostasis and oxidative stress, and construct a miRNA–mRNA regulatory network to identify central hub genes and regulatory miRNAs. This integrative approach may provide insight into ferroptosis regulation in TNBC and support the identification of potential biomarkers and therapeutic targets.

KEY MESSAGES

- TNBC exhibits a distinct "iron-hoarding" transcriptomic profile characterized by marked FTMT upregulation and SLC40A1 downregulation, which disrupt iron homeostasis and increase cellular vulnerability to ferroptosis.
- Regulatory Hubs: Multi-omics integration identified NQO1 and SLC38A1 as central mRNA hubs within a complex miRNA–mRNA network governing the ferroptosis-related regulatory landscape of triple-negative tumors.
- Although many mRNAs demonstrate prognostic potential in univariate analyses, only hsa-miR-378c and hsa-miR-449a emerged as robust independent prognostic biomarkers, offering improved risk stratification for patients with TNBC.

METHODS

Study Design

This study was designed as an integrative bioinformatics analysis of multiple publicly available transcriptomic and proteomic datasets.

Data Sources and Preprocessing

In this study, TCGA-BRCA served as the primary exploratory cohort. RNA sequencing (mRNA), miRNA sequencing profiles, and corresponding clinical data were retrieved from TCGA through the Genomic Data Commons (GDC), which includes patients enrolled between 2006 and 2013. The cohort initially included 1,087 primary breast tumor samples. Patients were classified into TNBC and non-TNBC groups according to the PAM50 intrinsic subtype, with the basal-like subtype defined as TNBC. After quality control and the exclusion of samples with incomplete clinical or molecular data, the final cohort comprised 192 TNBC and 895 non-TNBC cases.

The METABRIC dataset included patients enrolled between 1977 and 2005, comprising 309 TNBC and 1,455 non-TNBC cases, and served as an independent validation cohort for the mRNA-level findings.

Proteomic profiles from the TCGA reverse-phase protein array (RPPA) dataset, which included samples collected between 2011 and 2015, were incorporated to evaluate the protein-level expression of selected ferroptosis-related genes.

The final sample sizes represented the maximum numbers of cases with complete molecular and clinical data after quality control.

Differential Expression Analysis

Differential expression analysis was performed to identify ferroptosis-related mRNAs, proteins, and regulatory miRNAs that were significantly altered between the TNBC and non-TNBC subtypes. For the transcriptomic data, mRNA-seq and miRNA-seq profiles were analyzed using the DESeq2 package with a negative binomial generalized linear model. Differential expression was defined as a false discovery rate

(FDR)-adjusted p-value (padj)<0.05 and an absolute log2 fold change (|log2FC|)>1. Proteomic data were evaluated using the limma package. Because protein expression has a smaller dynamic range than transcript expression, significant protein alterations were defined as padj<0.05 and |log2FC|>0.5. No additional covariates were included in the primary models because preliminary inspection indicated minimal batch effects. The results were visualized using volcano plots, and differentially expressed molecules were prioritized for downstream functional annotation and network integration.

Functional Enrichment and Pathway Analyses

A comprehensive Gene Ontology (GO) enrichment analysis focusing on biological processes, molecular functions, and cellular components was performed to elucidate the biological roles of the identified ferroptosis-associated miRNAs. Accordingly, the target genes of significant miRNAs were retrieved from the multiMiR database, and gene identifiers were converted from Ensembl IDs to Entrez IDs using the bitr function in the clusterProfiler R package. GO biological process, molecular function, and cellular component analyses were then performed to identify overrepresented biological functions and map the functional architecture and regulatory effects of the miRNA–target interactome across the cellular hierarchy, with enrichment significance defined as p<0.05 after correction for multiple testing.

In parallel, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed on the same gene sets using clusterProfiler to identify enriched pathways associated with ferroptosis, oxidative stress, glutathione metabolism, and other cancer-related signaling processes.

Construction and Analysis of the miRNA–mRNA Regulatory Network

To ensure the biological accuracy of the regulatory landscape, the transcriptomic data were first standardized. Because miRNA expression was initially retrieved at the precursor miRNA (pre-miRNA) level, the precursors were mapped to their corresponding mature sequences using miRBase version 22 annotations. This mapping was critical because mature miRNA strands, rather than their precursors, mediate interactions with mRNAs. All downstream analyses, including target prediction and binding-site classification, were performed using these biologically active mature sequences (Supplementary Table 1).

The network was constructed using only significantly differentially expressed miRNAs (padj<0.05, |log2FC|>1) and significantly differentially expressed ferroptosis-related genes. Interactions were retrieved from miRDB, TargetScan version 8.0, and miRTarBase using the multiMiR package and were included if they were either experimentally validated in miRTarBase or predicted by both TargetScan version 8.0 and miRDB. This process generated a consolidated interaction matrix that was used to construct a directed network in which edges extended from miRNAs to their target genes.

The resulting network was imported into Cytoscape for visualization and topological analysis, and node-level topological parameters, including degree, betweenness centrality, and closeness centrality, were calculated to identify highly connected nodes within the network. High-degree nodes representing hub miRNAs or genes were prioritized as potential key regulators. To assess their biological relevance, these hub molecules were cross-referenced with the published literature on breast cancer progression and ferroptosis regulation, enabling the identification of candidate biomarkers and potential therapeutic targets.

To validate the regulatory potential of the identified hubs, Spearman correlation analysis was performed to evaluate the relationships between the expression levels of miRNAs and their target mRNAs across the dataset. Finally, detailed molecular binding metrics were retrieved for the most critical interactions. These metrics included target-binding regions, seed-match types, and site-conservation status identified using TargetScan and miRDB. Standard nomenclature, including miRBase accession numbers and strand specifications, was maintained throughout the analysis to ensure the full reproducibility of the regulatory model.

Statistical Analysis and Prognostic Modeling

All computational analyses were conducted in the R programming environment, version 4.3.2, using Bioconductor packages. Differential expression analysis was performed using the DESeq2 package, whereas functional enrichment analyses and network visualizations were generated using clusterProfiler, ggplot2, and Cytoscape.

To evaluate the clinical relevance of the identified molecules, Kaplan–Meier curves and univariate Cox regression analyses were used to compare overall survival between the high- and low-expression groups for both mRNAs and miRNAs. The prognostic value of these biomarkers was further assessed using multivariable Cox proportional hazards models, which estimated hazard ratios (HRs) and 95% confidence intervals (CIs). These models were adjusted for clinically relevant covariates, including age and pathological stage, to determine independent prognostic significance. For all statistical tests, p<0.05 was considered statistically significant.

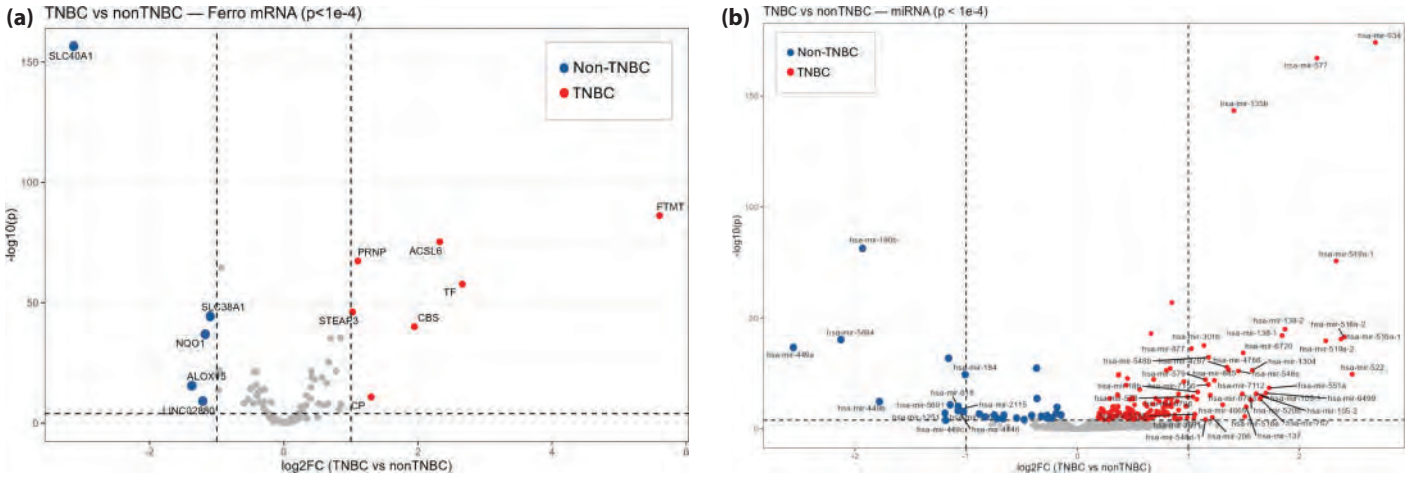

Figure 1. Differential expression of ferroptosis-related mRNAs and miRNAs between TNBC and non-TNBC.

RESULTS

Differential Expression of Ferroptosis-Related Transcripts and Proteins

A curated set of 259 ferroptosis-related genes was compiled from the FerrDb v2 database and recent literature, encompassing known drivers, suppressors, and markers of ferroptotic cell death.[12] Differential gene expression analysis identified several mRNAs with significant expression changes between the TNBC and non-TNBC groups (Fig. 1a). Among the significantly upregulated genes, FTMT ($log_2FC=5.60$, padj<0.001) exhibited the greatest fold increase. Other notably upregulated genes included TF ($log_2FC=2.66$, padj<0.001), ACSL6 ($log_2FC=2.32$, padj<0.001), CBS ($log_2FC=1.95$, padj<0.001), CP ($log_2FC=1.30$, padj<0.001), STEAP3 ($log_2FC=1.02$, padj<0.001), and PRNP ($log_2FC=1.10$, padj<0.001). Conversely, several genes were significantly downregulated, including SLC40A1 ($log_2FC=-3.13$, padj<0.001), SLC38A1 ($log_2FC=-1.11$, padj<0.001), NQO1 ($log_2FC=-1.18$, padj<0.001), ALOX15 ($log_2FC=-1.37$, padj<0.001), and LINC02880 ($log_2FC=-1.20$, padj<0.001).

Differential expression analysis identified 197 miRNAs with statistically significant expression changes between the TNBC and non-TNBC groups (Fig. 1b). A predominantly upregulated pattern was observed, with most significant miRNAs exhibiting positive log_2FC values, suggesting increased miRNA transcriptional activity. Several miRNAs were markedly upregulated. Among these, hsa-miR-135b ($log_2FC=1.41$, padj<0.001), hsa-miR-138-1 ($log_2FC=1.84$, padj<0.001), and hsa-miR-138-2 ($log_2FC=1.87$, padj<0.001) exhibited the most pronounced increases. Other substantially upregulated miRNAs included hsa-miR-130b, hsa-miR-18a, hsa-miR-4766, hsa-miR-188, hsa-miR-3200, hsa-miR-224, and hsa-miR-301b, all of which exhibited padj<0.001 and log_2FC values ranging from 0.32 to 1.36. Conversely, several miRNAs were significantly downregulated. hsa-miR-449a ($log_2FC=-2.56$, padj<0.001) and hsa-miR-190b ($log_2FC=-1.93$, padj<0.001) exhibited the greatest reductions, identifying them as key downregulated regulators. Other markedly downregulated miRNAs included hsa-miR-449b, hsa-miR-449c, hsa-miR-184, hsa-miR-135a-1, hsa-miR-135a-2, and hsa-miR-375 (log_2FC range, -1.78 to -0.60; padj<0.001).

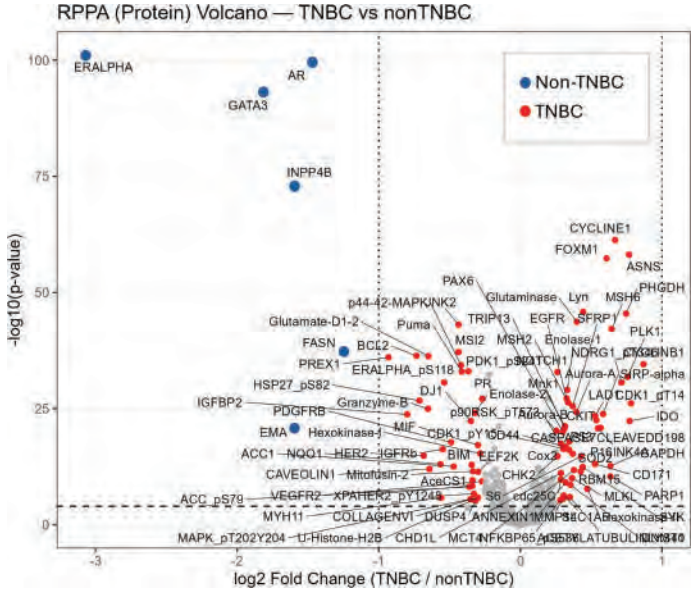

Figure 2. Differential expression of ferroptosis-related proteins between TNBC and non-TNBC.

Reverse-phase protein array analysis of 102 proteins revealed distinct protein expression patterns between TNBC and non-TNBC tumors (Fig. 2). Notably, hormone receptor-related

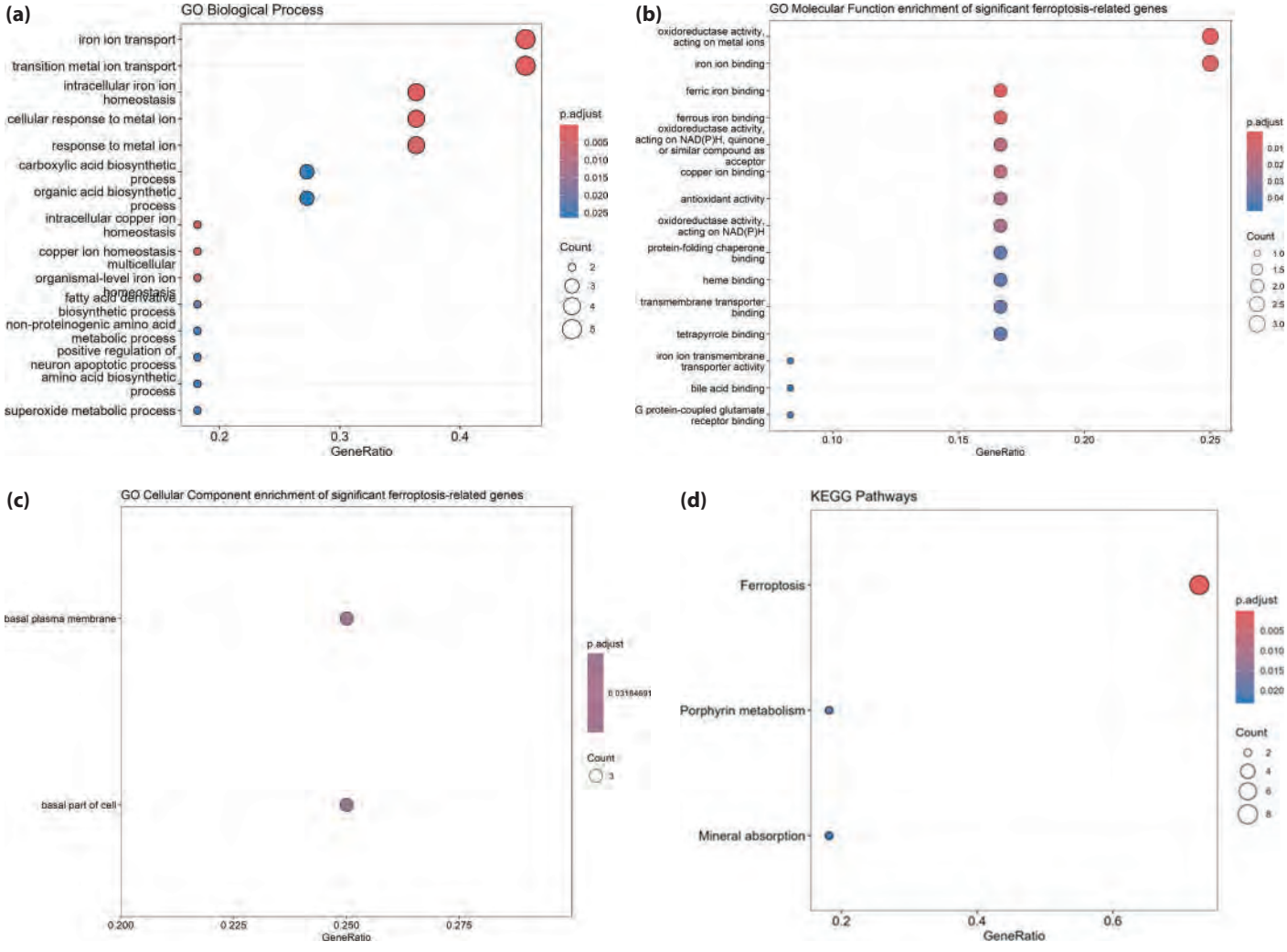

Figure 3. Gene ontology biological process enrichment dot plot for ferroptosis target genes. The y-axis represents the biological processes in which ferroptosis target genes were significantly enriched. The x-axis indicates the GeneRatio, defined as the proportion of analyzed genes associated with a given GO term relative to the total number of genes in that term. Dot size reflects the number of target genes associated with each GO term, whereas color intensity represents the adjusted p-value (padj), with red indicating lower and more statistically significant values.

proteins, including ERALPHA (logFC=-3.07, padj<0.001), AR (logFC=-1.48, padj<0.001), and GATA3 (logFC=-1.82, padj<0.001), were significantly downregulated in TNBC. Conversely, TNBC samples exhibited significant upregulation of proteins associated with cell-cycle regulation, proliferation, and metabolic adaptation. Among these, CYCLIN E1 (logFC=0.67, padj<0.001), ASNS (logFC=0.77, padj<0.001), FOXM1 (logFC=0.61, padj<0.001), PHGDH, MSH6, and CYCLIN B1 were prominently elevated. Altered expression of apoptosis-related proteins, including BCL2, PUMA, and caspase-7, and signaling proteins, such as p44-42-MAPK, PDK1, and IGF1R, further delineated the aggressive phenotype of TNBC.

Transcriptomic Enrichment of Ferroptosis-Related Processes

Functional enrichment analysis revealed a striking convergence of biological processes across both the mRNA and miRNA regulatory layers, primarily centered on iron metabolism and redox homeostasis (Fig. 3a). Gene Ontology analysis of differentially expressed mRNAs showed nominal enrichment in processes including monoatomic cation transport (p=0.044), metal ion transport (p=0.044), and iron ion transport (p=0.045), involving key ferroptosis-related genes such as CP, TF, SLC40A1, and FTMT. Moreover, SLC38A1 was identified as a key participant in glutamine transport;

given that glutaminolysis is a primary driver of ferroptosis, its involvement highlights the metabolic reprogramming that supports ferroptotic vulnerability in TNBC. However, although these transcriptomic changes were subtle and did not remain significant after correction for multiple testing, they were strongly reflected in the regulatory potential of the identified miRNAs. GO enrichment analysis of the target genes regulated by ferroptosis-associated miRNAs revealed an even more robust signature. The most significantly enriched process was iron ion transport (GO:0006826; padj<0.001), involving many of the same core genes identified in the mRNA analysis, including CP, TF, SLC40A1, and FTMT, along with STEAP3. Closely related processes, such as transition metal ion transport (GO:0000041) and intracellular iron ion homeostasis (GO:0006879), were also highly represented, further supporting the involvement of these miRNA targets in metal ion regulation.

Furthermore, both datasets highlighted a coordinated response to oxidative stress and lipid processing. miRNA targets were significantly enriched in the cellular response to metal ions (GO:0071248) and the response to oxidative stress, implicating genes such as ALOX15, PRNP, and NQO1.

Functional Profiling and Pathway Validation of the Ferroptosis-Associated Proteome

GO enrichment analysis of the RPPA dataset revealed a broad spectrum of significantly overrepresented biological processes, with a strong emphasis on cellular stress responses, cell death, and immune regulation. The most significantly enriched terms were related to the intrinsic apoptotic signaling pathway (GO:0097193, padj<0.001), signal transduction in response to DNA damage (GO:0042770, padj<0.001), and their intersection in the DNA damage-induced intrinsic apoptotic pathway (GO:0008630, padj<0.001). The analysis also highlighted a major role for p53-mediated signaling (GO:0072331) and cell-cycle checkpoint control (GO:0000077), underscoring the importance of maintaining genomic integrity. Concurrently, robust enrichment was observed in processes governing immune cell activation and differentiation, particularly those involving T cells (GO:0050863) and lymphocytes, as well as the regulation of cell-cycle phase transitions (GO:1901987). Key regulatory proteins, such as TP53, BCL2, ATM/ATR, and components of the mTOR and EGFR signaling pathways, were recurrently identified as central nodes across these critical processes, suggesting that they are pivotal integrators of apoptotic, DNA damage-related, and proliferative signals within the dataset.

Molecular Function and Cellular Component Analyses of Ferroptosis Regulators

Molecular function (MF) enrichment analysis further elucidated the biochemical activities underlying the ferroptotic landscape of TNBC (Fig. 3b). The most significant enrichment was observed in oxidoreductase activity acting on metal ions and iron ion binding (padj=0.01), specifically involving both ferric and ferrous forms of iron. This finding was complemented by significant enrichment in iron ion transmembrane transporter activity, providing a molecular basis for the systemic alterations in iron metabolism observed in the pathway analysis. Additionally, the identification of antioxidant activity and oxidoreductase activity acting on NAD(P)H (padj=0.03) underscored the disruption of redox-balancing mechanisms. Notably, the enrichment of G protein-coupled glutamate receptor binding (padj=0.04) provided molecular-level support for the involvement of glutaminolysis pathways, as highlighted by the dysregulation of SLC38A1. Furthermore, cellular component (CC) enrichment analysis identified the basal plasma membrane and basal region of the cell (padj=0.03) as the primary sites of dysregulation (Fig. 3c).

KEGG Pathway Enrichment and Functional Mapping of Ferroptosis Signaling

Functional characterization using KEGG pathway analysis revealed a high concentration of differentially expressed genes within the ferroptosis pathway, which emerged as the most statistically significant term (padj<0.001) (Fig. 3d). This enrichment was characterized by an exceptionally high fold enrichment of 163.58, with 8 of the 11 input genes, including CP, TF, and SLC40A1, mapping directly to this cell-death mechanism. Beyond the core ferroptosis machinery, the analysis identified significant enrichment in porphyrin metabolism (padj=0.019) and mineral absorption (padj=0.023).

Construction and Analysis of Regulatory Networks

The constructed miRNA–gene regulatory network revealed a sparse but modular topology, consisting of miRNAs with limited but specific connections to their target genes (Fig. 4). Topological analysis of the miRNA–gene regulatory network, comprising 19 nodes and 15 edges, revealed a hub-driven structure with a network density of 0.0439 and an average degree of 1.58. Network centrality analysis (Supplementary Table 2) indicated that hsa-miR-449a had the highest degree (2) and closeness centrality (0.75) among the miRNAs, suggesting a relatively central regulatory role. In contrast, hsa-miR-618 and hsa-miR-421 exhibited high closeness centrality values (1.0) despite having only one connection, implying direct regulation of highly central genes. Among the target genes, NQO1 and SLC38A1 emerged as hub nodes (degree=4; closeness centrality=0.357), representing major integration points of miRNA regulation. Other genes, such as STEAP3 and ALOX15, showed moderate connectivity, whereas ACSL6 and SLC40A1 exhibited high closeness centrality values (1.0), indicating topological importance despite having few interactions.

Figure 4. Constructed miRNA–gene regulatory network.

Furthermore, Spearman correlation analysis was performed between the expression levels of the miRNAs and their target mRNAs to validate the predicted miRNA–mRNA interactions. Significant negative correlations were observed, supporting the suppressive regulatory relationships of these pairs. Notably, hsa-miR-421 expression was strongly inversely correlated with SLC40A1 expression (Spearman r=-0.279, p<0.001). Other significant negative correlations included those between hsa-miR-378c and SLC38A1 (r=-0.130, p<0.001), hsa-miR-449a and STEAP3 (r=-0.087, p<0.001), hsa-miR-636 and SLC38A1 (r=-0.077, p<0.001), and hsa-miR-618 and ACSL6 (r=-0.070, p=0.023) (Table 1).

Integrated network and Spearman correlation analyses (Table 1) prioritized five key miRNA–mRNA regulatory pairs. For hsa-miR-421-5p and hsa-miR-449a-5p, the correlation-based evidence directly overlapped with the primary TargetScan predictions of SLC40A1 and STEAP3, respectively. However, for hsa-miR-378c and hsa-miR-636, network analysis identified SLC38A1 as the functionally relevant target in the context of TNBC, despite other genes having higher theoretical TargetScan scores (Table 2). All identified interactions were characterized by canonical seed matches within the 3′ untranslated regions of the target mRNAs, providing structural plausibility for the observed expression-based correlations.

mRNA and miRNA Survival and Cox Regression Analyses

Log-rank tests demonstrated significant differences in survival for SLC40A1 (p=0.034) and ALOX15 (p=0.016), whereas FTMT (p=0.46), NQO1 (p=0.67), SLC38A1 (p=0.19), and ACSL6 (p=0.076) did not reach statistical significance (Fig. 5a). In the univariate Cox regression analysis, SLC40A1 (HR=0.71, 95% CI: 0.51–0.98, p=0.035) and ALOX15 (HR=1.49, 95% CI: 1.08–2.07, p=0.017) were significantly associated with overall survival. Conversely, FTMT, NQO1, SLC38A1, and ACSL6 were not significantly associated with survival in the univariate analysis. However, after adjustment for age and pathological stage in the multivariable model, the prognostic significance of SLC40A1 (HR=0.73, 95% CI: 0.49–1.08, p=0.115) and ALOX15 (HR=1.17, 95% CI: 0.77–1.79, p=0.460) was no longer maintained.

Table 1. Spearman correlation analysis of predicted miRNA–mRNA interactions

miRNA	Target gene	Spearman correlation coefficient (r)	p
hsa-miR-421	SLC40A1	-0.279	<0.001
hsa-miR-378c	SLC38A1	-0.130	<0.001
hsa-miR-449a	STEAP3	-0.087	0.004
hsa-miR-636	SLC38A1	-0.077	0.011
hsa-miR-618	ACSL6	-0.070	0.023

The corresponding Kaplan–Meier analysis comparing the low- and high-miRNA-expression groups demonstrated significant differences in survival for hsa-miR-378c (p=0.0001), hsa-miR-449a (p=0.0159), and hsa-miR-3909 (p=0.018). The expression levels of hsa-miR-618 (p=0.101), hsa-miR-7706 (p=0.148), and hsa-miR-1270 (p=0.194) were not significantly associated with survival in the Kaplan–Meier analysis (Fig. 5b). Univariate Cox regression analysis indicated that hsa-miR-378c (HR=1.40, 95% CI: 1.00–1.96, p=0.050) and hsa-miR-449a (HR=0.59, 95% CI: 0.42–0.83, p=0.002) were significant prognostic factors, whereas hsa-miR-3909, hsa-miR-618, hsa-miR-7706, and hsa-miR-1270 were not. In the multivariable Cox regression analysis, both hsa-miR-378c (HR=1.56, 95% CI: 1.03–2.36, p=0.034) and hsa-miR-449a (HR=0.58, 95% CI: 0.38–0.86, p=0.008) remained statistically significant as independent predictors of survival.

DISCUSSION

A central finding of our analysis was the profound dysregulation of iron-handling genes, particularly the marked upregulation of mitochondrial ferritin (FTMT) and suppression of ferroportin (SLC40A1), the sole known cellular iron exporter. This "iron-hoarding" phenotype may, on the one hand, increase intracellular iron levels and promote TNBC progression by providing essential cofactors for enzymes such as ribonucleotide reductase, which directly supports rapid DNA synthesis and tumor cell proliferation.[13,14] On the other hand, iron-dependent reactive oxygen species can activate

Table 2. Molecular characteristics of key predicted miRNA–mRNA interactions

Pre-miRNA (TCGA)	miRBase ID	Network- or correlation-supported target	TargetScan-listed target	Binding region	Seed-match type	Binding site	Prediction/support database	Notes*
hsa-miR-421	MIMAT0003339	SLC40A1	SLC40A1	3′ UTR	7mer-m8	Conserved site	TargetScan, miRDB	
hsa-miR-378c	MIMAT0016847	SLC38A1	PDIA4	3′ UTR	7mer-m8	Conserved site	TargetScan, miRDB	Family-based prediction (miR-378a-3p)
hsa-miR-636	MIMAT0003306	SLC38A1	CETN2	3′ UTR	8mer	Conserved site	TargetScan, miRDB	
hsa-miR-618	MIMAT0003287	ACSL6	LSM1	3′ UTR	7mer-m8	Conserved site	TargetScan, miRDB	
hsa-miR-449a	MIMAT0001541	STEAP3	MDM4	3′ UTR	8mer	Conserved site	TargetScan, miRDB	Predicted through the miR-34 family (representative miRNA: miR-34c-5p)

prosurvival pathways, such as NF-κB and HIF-1α.[15,16] Our results suggest that TNBC exists on a metabolic knife-edge: although iron fuels its aggressive growth, it simultaneously sensitizes tumor cells to lipid peroxidation, thereby creating a targetable vulnerability unique to this subtype.

Our integrative network analysis revealed that this iron-sequestering state is not merely a passive byproduct of cancer but is actively maintained through post-transcriptional regulation. Specifically, the significant inverse correlation between hsa-miR-421 and SLC40A1 suggests that impaired iron export is actively maintained through miRNA-mediated silencing. This regulatory axis identifies hsa-miR-421 as a key driver of the iron-rich phenotype. Furthermore, the identification of hsa-miR-449a as a central regulatory hub is particularly important. Loss of this tumor-suppressor miRNA family may derepress proferroptotic metabolic drivers and oncogenic pathways, further exacerbating the oxidative vulnerability of TNBC cells.[17–20]

Furthermore, we identified SLC38A1, a major glutamine transporter, as a central hub within the regulatory network. Because glutaminolysis is a major driver of ferroptosis, dysregulation of SLC38A1 highlights the metabolic alterations that support ferroptotic vulnerability. This finding was mirrored at the protein level, where TNBC samples exhibited significant upregulation of metabolic adaptation proteins, including PHGDH and ASNS.[21,22] This intersection between nutrient transport and oxidative stress defense demonstrates how the metabolic flexibility of TNBC is structurally supported by its miRNA–mRNA regulatory network.

Although iron-handling genes such as SLC40A1 and ALOX15 demonstrated prognostic significance in univariate analyses, they lost their independent significance after adjustment for clinical covariates, including age and pathological stage. In contrast, hsa-miR-378c and hsa-miR-449a remained independent predictors of survival, suggesting that miRNAs may provide a more integrated and stable representation of tumor behavior than individual mRNA expression levels.

Several limitations should be considered when interpreting our findings. The differential expression analysis was not adjusted for important clinical covariates, which may have introduced confounding effects. In addition, the integration of multiple datasets may have introduced technical batch effects despite normalization procedures. The miRNA–mRNA regulatory networks were primarily based on in silico predictions and correlation analyses without direct experimental validation. Dataset limitations also prevented differentiation between mature miRNA strands (-5p/-3p), resulting predominantly in family-level analyses. Furthermore, the multivariable survival models included only age and tumor stage because of limited clinical annotations. Therefore, our findings should be considered

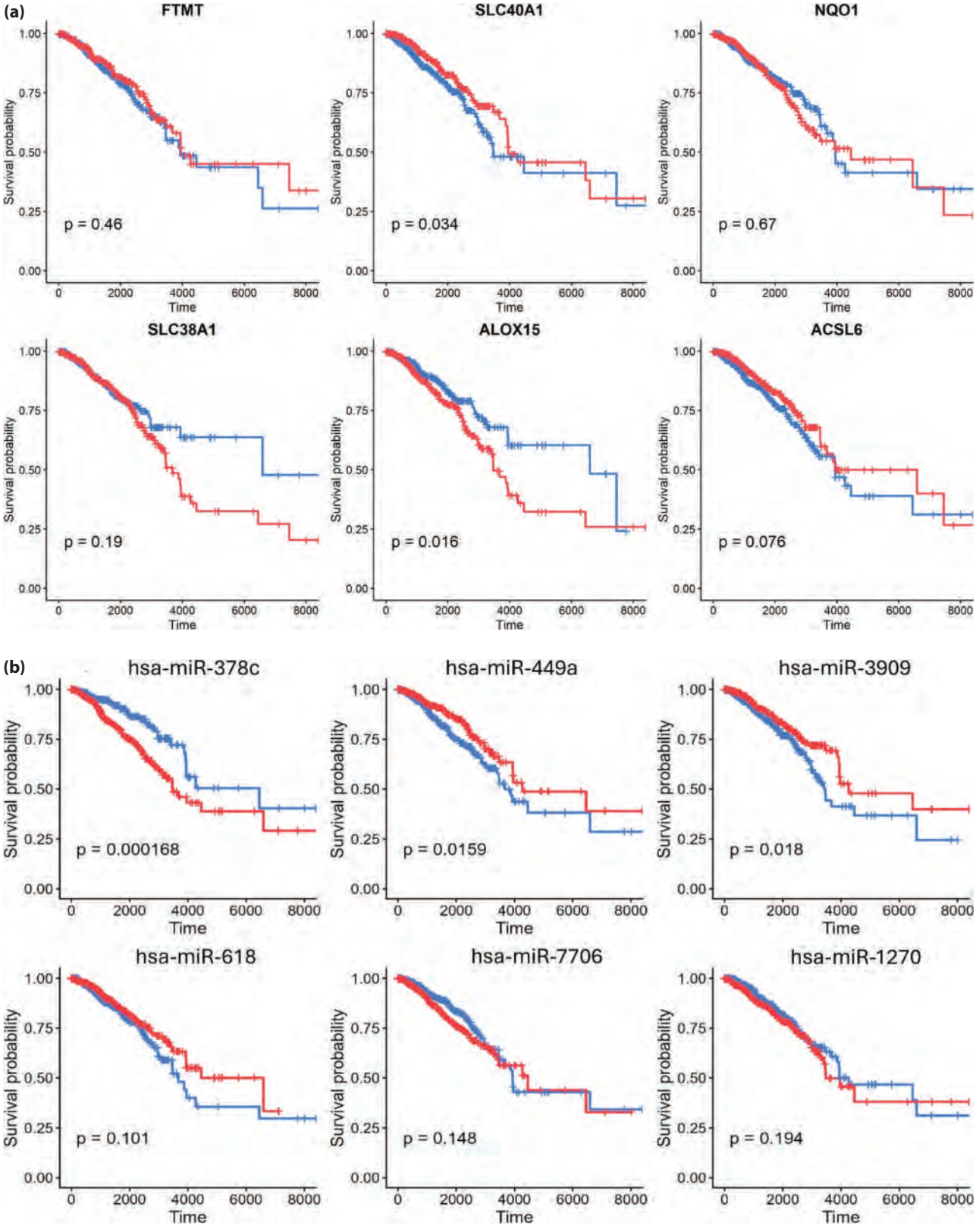

Figure 5. Kaplan–Meier survival curves for hub ferroptosis-related mRNAs and miRNAs in TNBC.

hypothesis-generating and require further in vitro and in vivo validation to confirm their functional relevance in TNBC.

CONCLUSION

This study comprehensively characterized the ferroptosis-related miRNA–mRNA regulatory landscape in TNBC. Although genes such as NQO1, SLC38A1, and SLC40A1 emerged as key regulatory nodes, only hsa-miR-378c and hsa-miR-449a demonstrated independent prognostic value in multivariable survival analyses. These miRNAs may serve as promising biomarkers for risk stratification, whereas the broader mRNA network may provide a framework for developing ferroptosis-targeted therapies in TNBC.

Ethics Committee Approval: Ethics committee approval was not required, as this study was designed as a multi-dataset, integrative bioinformatics analysis using publicly available transcriptomic and proteomic data.

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Supplementary Table 1. Standardized nomenclature and annotations of the analyzed miRNAs

Pre-miRNA	Mature miRNA	miRBase accession number	Strand specification
hsa-mir-421	hsa-miR-421	MIMAT0002873	5p dominant
hsa-mir-449a	hsa-miR-449a-5p	MIMAT0001541	5p
hsa-mir-378c	hsa-miR-378c	MIMAT0004957	3p/5p reported
hsa-mir-636	hsa-miR-636	MIMAT0003304	Unclear
hsa-mir-618	hsa-miR-618	MIMAT0003280	5p dominant

This table provides complete standardized nomenclature, including miRBase accession numbers and strand dominance, to ensure precise identification and reproducibility.

Supplementary Table 2. Topological centrality metrics of the miRNA–mRNA regulatory network

Node type	Node name	Degree	Betweenness centrality	Closeness centrality
Gene	NQO1	4	0	NA*
Gene	SLC38A1	4	0	NA*
Gene	ALOX15	2	0	NA*
Gene	STEAP3	2	0	NA*
Gene	ACSL6	1	0	NA*
Gene	CP	1	0	NA*
Gene	SLC40A1	1	0	NA*
miRNA	hsa-miR-3662	2	0	0.5
miRNA	hsa-miR-940	2	0	0.5
miRNA	hsa-miR-449a	2	0	0.5
miRNA	hsa-miR-618	1	0	1.0
miRNA	hsa-miR-1270	1	0	1.0
miRNA	hsa-miR-3909	1	0	1.0
miRNA	hsa-miR-7706	1	0	1.0
miRNA	hsa-miR-2110	1	0	1.0
miRNA	hsa-miR-378c	1	0	1.0
miRNA	hsa-miR-636	1	0	1.0
miRNA	hsa-miR-421	1	0	1.0
miRNA	hsa-miR-184	1	0	1.0

This table presents node-level quantitative metrics, including degree, betweenness centrality, and closeness centrality, demonstrating the hierarchical and hub-driven structure of the network. *Closeness centrality for target gene nodes is reported as NA (not applicable) because of the directed, bipartite structure of the network from miRNAs to mRNAs.

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HIV Seroprevalence and Screening Practices Among Cancer Patients Receiving Immune Checkpoint Inhibitors: A Single-Center Study From Türkiye

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ABSTRACT

Objective: HIV infection affects the immune system and may influence the safety and efficacy of immune checkpoint inhibitors (ICIs). This study aimed to evaluate HIV seroprevalence among cancer patients receiving ICIs and contextualize the findings using national prevalence estimates.

Materials and Methods: We retrospectively analyzed 590 patients treated with ICIs between January 2015 and July 2025. Viral serology, including anti-HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV) markers, was evaluated. HIV positivity was defined based on confirmatory testing. Statistical analyses were performed using SPSS, version 27.

Results: The median age was 65 years (IQR, 55–73 years), and 65.9% of the patients were male. The most common diagnoses were melanoma (37.4%), genitourinary tumors (26.4%), and lung cancer (21.5%). Viral serology results were available for 354 patients (60%). Only one patient had confirmed HIV positivity, corresponding to a prevalence of 0.28% (exact 95% CI, 0.007–1.56%). This patient had metastatic melanoma and tested negative for HBV and HCV. The observed point estimate was numerically similar to the reported national adult HIV prevalence estimates in Türkiye (0.1–0.2%). Patients tested for HIV were younger and more likely to have comorbidities, and the distribution of tumor types differed between tested and untested patients. In the multivariable analysis, younger age, the presence of comorbidities, and tumor type were independently associated with HIV testing.

Conclusion: HIV prevalence among cancer patients treated with ICIs was low. Given the incomplete testing coverage and the clinical implications of undiagnosed HIV infection, routine pretreatment HIV screening should be incorporated into standard viral serology panels.

Keywords: HIV, immune checkpoint inhibitors, screening strategies, seroprevalence, solid tumors.

INTRODUCTION

Immune checkpoint inhibitors (ICIs) are a foundational class of therapies that prolong survival in patients with various solid tumors. The modulation of immune responses by ICIs increases the clinical significance of underlying viral infections. HIV infection represents a distinct clinical consideration because of its immunosuppressive effects and its potential to modify the toxicity profile associated with immunotherapy. Targeting the PD-1/PD-L1 axis may also enhance HIV-specific T-cell responses and affect the latent viral reservoir.[1] Combination antiretroviral therapy (ART) has transformed HIV infection into a chronically manageable condition. However, people living with HIV (PLWH), whose life expectancy has increased, remain at elevated risk of both AIDS-defining and non-AIDS-defining malignancies. Large cohort studies indicate that approximately 9% of HIV-positive individuals develop cancer.[2,3] Many studies examining HIV prevalence among patients with cancer include only those with a known HIV diagnosis. Consequently, these studies may underestimate the actual prevalence.[4]

The World Health Organization (WHO) and European guidelines classify specific malignancies and conditions requiring immunosuppressive therapy as "indicator conditions" for HIV testing and recommend routine testing in these settings.[5–7] In healthcare centers without systematic screening programs, undiagnosed HIV infections are more likely to remain undetected.

Although the prevalence of HIV among adults in Türkiye is relatively low, at approximately 0.1–0.2%, according to national and international surveillance reports,[8–11] patients receiving systemic anticancer therapy are more susceptible to infections because of treatment-related and disease-related immunosuppression. National epidemiological studies and temporal trend analyses consistently indicate that HIV prevalence in Türkiye remains low in the general population compared with global averages.[9,12] Establishing baseline HIV status in patients scheduled to receive immunotherapy is critical for effective toxicity management and informed clinical decision-making. This study aimed to determine the seroprevalence of HIV among patients with cancer receiving immunotherapy and to compare the findings with national prevalence data, thereby highlighting the importance of incorporating HIV testing into routine pre-immunotherapy assessments. In addition, we aimed to evaluate the rate of HIV testing in routine clinical practice before or during ICI therapy.

METHODS

This retrospective, single-center observational study was conducted at the Department of Medical Oncology, Faculty of Medicine, Ege University, a tertiary referral center. Adult patients (≥18 years) with histologically confirmed solid tumors who received at least one cycle of ICI therapy, including anti–PD-(L)1 and/or CTLA-4 inhibitors, between January 2015 and July 2025 were included. Demographic characteristics, tumor type, disease stage at ICI initiation, treatment details, comorbidity status, and viral serology results were retrospectively collected from electronic medical records and institutional databases.

KEY MESSAGES

- HIV seroprevalence among cancer patients treated with ICIs was 0.28%, with one confirmed HIV-positive case among 354 tested patients.
- HIV testing results were available for only 60% of patients, and testing was associated with younger age, the presence of comorbidities, and tumor type.
- Routine HIV testing should be incorporated into standard viral screening panels before immunotherapy.

The study protocol was approved by Ege University Medical Research Ethics Committee (Approval Number: 26-2T/58, Date: 05.02.2026). Given the retrospective design and the use of anonymized data, the requirement for informed consent was waived. The study was conducted in accordance with the Declaration of Helsinki.

HIV seroprevalence was assessed only among patients with available anti-HIV test results from the institutional viral serology panel. The viral serology panel included HBsAg, anti-HBs, anti-HBc IgG, anti-HCV, and anti-HIV, and testing was performed using routine standardized laboratory assays. Reactive anti-HIV screening results were verified by Western blot, and HIV positivity was defined based on confirmatory testing.

A total of 590 patients treated with ICIs were identified. Of these, 354 patients (60%) had available viral serology results and were included in the HIV seroprevalence analysis. Patients with and without available HIV test results were compared to evaluate potential selection bias associated with incomplete testing. For comparative and regression analyses, disease stage was categorized as stages I–III or stage IV. Tumor types were grouped as lung cancer, genitourinary tumors, melanoma, and other tumors. Comorbidity status was categorized as present or absent according to documentation in the electronic medical records.

Statistical analyses were performed using IBM SPSS Statistics, version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as the median (interquartile range [IQR]), and categorical variables were summarized as numbers

Table 1. Comparison of patients with and without HIV testing

Variable	Subgroup	HIV Testing performed (n=354)	HIV testing not performed (n=236)	p
Age, years	Median (IQR)	61.3 (22.0)	67.3 (16.7)	<0.001
Sex	Male	233 (65.8)	156 (66.1)	0.891
	Female	121 (34.2)	80 (33.9)	
Comorbidity	Present	167 (47.2)	82 (34.7)	0.003
	Absent	187 (52.8)	154 (65.3)	
Disease stage	Stage I–III	158 (44.6)	105 (44.5)	0.991
	Stage IV	196 (55.4)	131 (55.5)	
Tumor type	Lung cancer	79 (22.3)	48 (20.3)	0.037
	Genitourinary tumors	79 (22.3)	77 (32.6)	
	Melanoma	139 (39.3)	82 (34.7)	
	Other tumors	57 (16.1)	29 (12.3)	

Data are presented as the median (IQR) or n (%). P values were calculated using the Mann–Whitney U test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables, as appropriate. IQR: Interquartile range.

and percentages. HIV seroprevalence was calculated as point prevalence with exact 95% confidence intervals (CIs) using the Clopper–Pearson method. The observed seroprevalence was descriptively compared with the reported national adult HIV prevalence estimates in Türkiye (0.1–0.2%).[11] To identify factors associated with the availability of HIV testing, univariable logistic regression analyses were initially performed. Variables with p<0.20 in the univariable analyses were subsequently included in the multivariable logistic regression model. Results were reported as odds ratios (ORs) with 95% CIs. Tumor type was analyzed as a categorical variable, with the "other tumors" group serving as the reference category. A two-sided p<0.05 was considered statistically significant.

RESULTS

Baseline Characteristics and Treatment Exposure of the Study Cohort

A total of 590 patients treated with immunotherapy were included. The median age was 65 years (IQR, 55–73 years), and 389 patients (65.9%) were male. Melanoma was the most common diagnostic group (37.4%), followed by genitourinary tumors (26.4%) and lung cancer (21.5%). At the initiation of immunotherapy, 327 patients (55.4%) had stage IV disease, whereas 263 patients (44.6%) had stage I–III disease. Regarding treatment exposure, nivolumab was the most frequently administered agent (72.5%), followed by pembrolizumab (15.3%) and atezolizumab (5.1%).

Baseline characteristics according to the availability of HIV testing are presented in Table 1. Patients who underwent HIV testing were significantly younger than those who did not undergo testing (median age, 61.3 vs 67.3 years; p<0.001). Patients with comorbidities were also more likely to undergo HIV testing (47.2% vs 34.7%; p=0.003). No significant differences were observed with respect to sex or disease stage. The distribution of tumor types differed significantly between the groups (p=0.037), with genitourinary tumors being more common among patients who did not undergo HIV testing.

Availability of Viral Serology and HIV Seroprevalence

Among the 590 patients included in the study, viral serology results were available for 354 patients (60%), whereas no serological data were available for 236 patients (40%). Therefore, the HIV seroprevalence analysis was performed exclusively among the 354 patients with available viral serology results.

Of the 354 patients who underwent anti-HIV testing, only one had confirmed HIV positivity. This patient had advanced-stage melanoma and no concomitant HBV or HCV infection. Accordingly, the HIV seroprevalence was 0.28% (1/354), with an exact 95% confidence interval of 0.007–1.56% based on the Clopper–Pearson method. The observed point estimate of 0.28% was numerically similar to the reported national adult HIV prevalence estimates in Türkiye of 0.1–0.2% (Fig. 1).[11,13]

HBV and HCV serology results were also evaluated as part of the institutional viral screening panel. Among the 354 patients with available viral serology results, HBsAg positivity was detected in 14 patients (4.0%), whereas no patients had anti-HCV positivity. Anti-HBs positivity was observed in 154 patients (43.5%). Serological evidence of resolved HBV infection with acquired immunity, defined as concomitant anti-HBc IgG and anti-HBs positivity, was present in 57 patients (16.1%). Vaccine-induced immunity, defined as isolated anti-

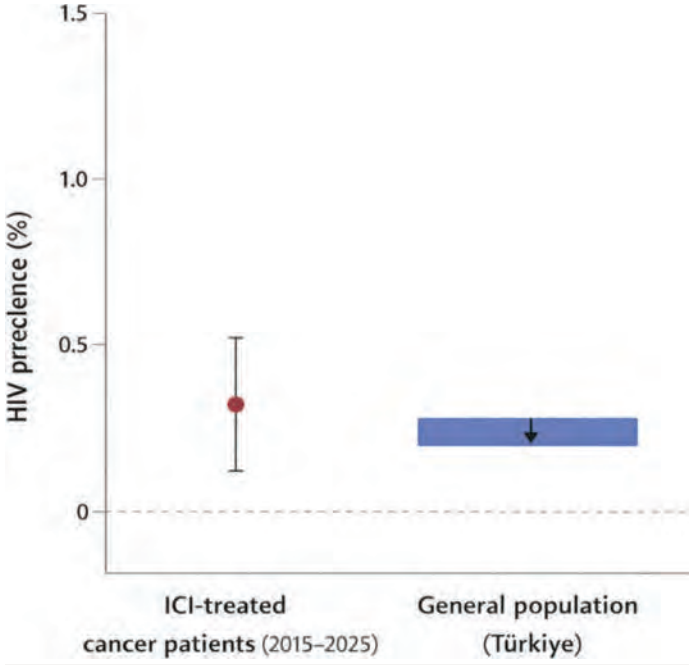

Figure 1. Comparison of HIV prevalence between cancer patients treated with ICIs and the general adult population in Türkiye. The point estimate of HIV prevalence among cancer patients treated with ICIs is presented with the exact 95% confidence interval calculated using the Clopper–Pearson method and compared with the estimated HIV prevalence range in the general adult population in Türkiye (0.1–0.2%).

HBs positivity, was observed in 97 patients (27.4%). Isolated anti-HBc IgG positivity was detected in 32 patients (9.0%). These viral serology results are summarized in Table 2.

Factors Associated With HIV Testing Availability

To further evaluate potential selection bias resulting from incomplete testing, univariable and multivariable logistic regression analyses were performed to identify factors associated with the availability of HIV testing. In the univariable analyses, younger age (OR, 0.972; 95% CI, 0.960–0.985; p<0.001), the presence of comorbidities (OR, 1.666; 95% CI, 1.186–2.341; p=0.003), and tumor type (overall p=0.038) were significantly associated with HIV testing. Sex (OR, 0.972; 95% CI, 0.650–1.453; p=0.891) and disease stage (OR, 1.002; 95% CI, 0.719–1.396; p=0.991) were not associated with testing practices.

Variables with p<0.20 in the univariable analyses were subsequently included in the multivariable logistic regression model. In the multivariable analysis, younger age remained independently associated with a higher likelihood of HIV testing (OR, 0.966; 95% CI, 0.953–0.980; p<0.001). Patients with comorbidities were also more likely to undergo HIV testing (OR, 1.908; 95% CI, 1.338–2.723; p<0.001). Tumor type remained independently associated with testing practices (overall p=0.011). Compared with the reference group of other tumor types, patients with genitourinary tumors were significantly less likely to undergo HIV testing (OR, 0.434; 95% CI, 0.244–0.772; p=0.005), whereas no significant differences were observed for patients with lung cancer (OR, 0.857; 95% CI, 0.470–1.562; p=0.614) or melanoma (OR, 0.616; 95% CI, 0.349–1.088; p=0.095) (Table 3).

Table 2. Viral serology results among patients with an available screening panel

Viral marker or serologic pattern	n (%)
Confirmed anti-HIV positivity	1 (0.28)
HBsAg positivity	14 (4.0)
Anti-HCV positivity	0 (0)
Anti-HBs positivity	154 (43.5)
Anti-HBc IgG and anti-HBs positivity	57 (16.1)
Isolated anti-HBs positivity	97 (27.4)
Isolated anti-HBc IgG positivity	32 (9.0)

Percentages were calculated among the 354 patients with available viral serology results. HIV positivity was defined only on the basis of confirmed positive results. Isolated anti-HBs positivity was considered consistent with vaccine-induced immunity, whereas concomitant anti-HBc IgG and anti-HBs positivity was considered consistent with resolved HBV infection and acquired immunity.

DISCUSSION

In this cohort, the HIV seroprevalence among patients receiving immunotherapy was 0.28%, which was numerically similar to national adult HIV prevalence estimates in Türkiye.[8,11] However, this comparison should be interpreted cautiously because the national estimates were surveillance-based, whereas our data were derived from a hospital-based oncology cohort with incomplete testing coverage. HIV testing results were available for only 60% of patients, and testing was associated with younger age, the presence of comorbidities, and tumor type, suggesting that screening appeared to be influenced by clinical practice patterns rather than being applied systematically. Therefore, the true HIV prevalence may have been underestimated. Nevertheless, the observed prevalence remained above the commonly cited threshold of 0.1% used to support routine HIV screening, underscoring the need for standardized pre-immunotherapy viral screening protocols.

The literature indicates that HIV prevalence in oncology populations is often underestimated.[14] Delayed diagnosis and the lack of routine screening in many centers contribute to this underestimation.[15] Previous studies from low- and middle-income countries have reported HIV prevalence rates of 2–10%

Table 3. Univariable and multivariable logistic regression analyses of factors associated with HIV testing availability

Variable	Univariable OR (95% CI)	p	Multivariable OR (95% CI)	p
Age, per 1-year increase	0.972 (0.960–0.985)	<0.001	0.966 (0.953–0.980)	<0.001
Male sex vs female sex	0.972 (0.650–1.453)	0.891	—	—
Stage IV disease vs stage I–III disease	1.002 (0.719–1.396)	0.991	—	—
Comorbidity present vs absent	1.666 (1.186–2.341)	0.003	1.908 (1.338–2.723)	<0.001
Tumor type, overall	—	0.038	—	0.011
Lung cancer vs other tumors	0.808 (0.454–1.440)	0.470	0.857 (0.470–1.562)	0.614
Genitourinary tumors vs other tumors	0.504 (0.291–0.874)	0.015	0.434 (0.244–0.772)	0.005
Melanoma vs other tumors	0.833 (0.491–1.412)	0.497	0.616 (0.349–1.088)	0.095

Variables with p<0.20 in the univariable analyses were included in the multivariable model. Age was analyzed as a continuous variable. Sex, disease stage, and comorbidity were coded as male versus female, stage IV versus stage I–III, and present versus absent, respectively. Tumor type was analyzed using other tumors as the reference category. CI: Confidence interval; OR: Odds ratio.

among patients with cancer, suggesting that HIV may be underrecognized in oncology populations.[16,17] Evidence from Asian countries also suggests that inadequate HIV screening practices and the absence of routine testing recommendations may contribute to the underdiagnosis of HIV among patients with cancer.[18,19] Consequently, undiagnosed HIV infections may persist in regions where screening strategies are inadequate. Similarly, studies from Europe and North America have shown that HIV testing rates remain suboptimal in oncology practice despite clinically relevant HIV prevalence among tested patients.[20–22] In one large cancer center cohort, HIV prevalence was 1.2% among patients tested before systemic therapy, exceeding the commonly cited threshold of 0.1% for routine screening.[22] These findings suggest that inadequate testing practices, rather than patient refusal alone, may contribute to the underdiagnosis of HIV in oncology populations.[4]

Historically, PLWH were systematically excluded from clinical trials evaluating ICIs. In numerous phase I–III studies, PLWH were either excluded entirely or conditionally included only if they met stringent criteria, such as an HIV RNA level below 50 copies/mL and a $CD4^+$ T-lymphocyte count above 200 cells/mm^3.[23] This exclusion substantially limited the available evidence regarding the use of immunotherapy in HIV-positive populations.

More recently, the growing availability of real-world data has begun to address these limitations. Puronen et al.[1] reviewed evidence indicating that PD-1/PD-L1 inhibitors demonstrate the expected antitumor efficacy in HIV-positive patients, particularly those with melanoma, lung cancer, and Hodgkin lymphoma, with an immune-related adverse event (irAE) profile similar to that observed in HIV-negative populations. Accumulating evidence, including multicenter studies, systematic reviews, and retrospective series, indicates that ICIs can be administered safely to patients with well-controlled HIV infection without adversely affecting $CD4^+$ T-lymphocyte counts, viral load, or other immunological parameters and with clinical outcomes broadly comparable to those observed in HIV-negative patients.[24–27] Therefore, well-controlled HIV infection should not be considered a contraindication to immunotherapy. Rather, the key clinical issue is the early identification of HIV infection before ICI initiation to facilitate appropriate infectious disease assessment, ART optimization, and multidisciplinary management.

ICIs function as potent immune activators and can induce autoimmune toxicities. In individuals with HIV infection, chronic immune dysfunction and latent infections may complicate the clinical presentation of these toxicities. In particular, in cases of undiagnosed HIV infection, distinguishing immune-related adverse events from opportunistic infections may be challenging. Consequently, determining HIV status before initiating immunotherapy is essential for accurately assessing potential toxicities and planning timely infection management.

For patients with confirmed HIV infection, current evidence suggests that concurrent ART and immunotherapy are generally safe. However, infectious disease and oncology teams should collaborate before treatment initiation, taking into account the $CD4^+$ T-lymphocyte count, viral load, comorbidities, and the need for prophylactic measures.[28] Current European and UK guidelines emphasize that HIV testing based solely on high-risk behavior is insufficient and recommend routine testing in patients with HIV indicator conditions, particularly in settings where the expected HIV prevalence exceeds 0.1%.[29–31]

Indicator condition–based testing strategies, including those evaluated in PROTEST and similar studies, have been shown to increase diagnostic yield and may be cost-effective; however, their implementation remains inconsistent.[32–34] These findings support systematic HIV assessment in oncology practice, particularly before chemotherapy or immunotherapy.

Several limitations should be considered. First, because of the retrospective design, HIV testing was not performed systematically, and anti-HIV results were available for only 60% of the cohort, raising concerns about selection bias and the potential underestimation of the true HIV prevalence. Although we performed additional comparative and multivariable analyses to identify factors associated with HIV testing, residual selection bias cannot be excluded. Second, the single-center design limits the generalizability of the findings to the broader oncology population in Türkiye. Third, the small number of HIV-positive cases precluded subgroup analyses of the safety and efficacy of ICIs in people living with HIV. The study was also underpowered to detect small differences relative to the population prevalence. Despite these limitations, this study provides the first large single-center experience from Türkiye evaluating HIV seroprevalence among cancer patients treated with ICIs and comparing it with national population estimates.

CONCLUSION

This study presents the first large single-center cohort from Türkiye evaluating HIV seroprevalence among cancer patients treated with ICIs and comparing it with national population estimates. HIV seroprevalence in this cohort was low at 0.28%. Although the point estimate was numerically similar to national adult prevalence estimates, the wide confidence interval, reflecting the small number of positive cases, precludes firm conclusions regarding comparability at the population level. Notably, the observed prevalence exceeded the commonly cited screening threshold of 0.1%. Given the potential clinical consequences of undiagnosed HIV infection during immunotherapy, these findings support incorporating HIV testing into routine pre-immunotherapy viral serology panels. Further multicenter studies with systematic testing are needed to better define HIV prevalence and optimize screening strategies in oncology practice.

Ethics Committee Approval: Ethics committee approval was obtained from Ege University Medical Research Ethics Committee (Approval Number: 26-2T/58, Date: 05.02.2026).

Informed Consent: Due to the retrospective design and use of anonymized data, informed consent was waived.

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Upadacitinib in Rheumatoid Arthritis After Prior TNF Inhibitor Failure: A 12-Month Multicenter Real-World Study

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ABSTRACT

Objective: Patients with rheumatoid arthritis who do not achieve an adequate response to tumor necrosis factor inhibitors remain difficult to manage in routine clinical practice. Data from daily clinical care regarding selective JAK1 inhibition in this setting are limited. This study aimed to evaluate the 12-month effectiveness and safety of upadacitinib in Turkish patients with rheumatoid arthritis and previous tumor necrosis factor inhibitor failure.

Materials and Methods: This retrospective multicenter study included adult patients with rheumatoid arthritis treated at two tertiary rheumatology centers who initiated upadacitinib at a dose of 15 mg/day after the failure of at least one tumor necrosis factor inhibitor. Disease activity, inflammatory markers, and functional status were assessed at baseline and at 3, 6, and 12 months using validated composite indices and patient-reported outcome measures. Changes over time were analyzed using repeated-measures statistical methods. Safety outcomes were collected from routine clinical follow-up records.

Results: Forty patients were included in the analysis, with a mean age of 48.4 years, and 62.5% were female. Consistent improvements were observed across disease activity measures throughout follow-up. At month 12, ACR20/50/70 response rates reached 87.5%, 77.5%, and 40.0%, respectively. Additionally, 45.0% of patients achieved low disease activity or remission according to the SDAI. Functional status improved in parallel with reductions in inflammatory and clinical parameters. No serious adverse events were observed during the 12-month follow-up, while the reported adverse events were mild.

Conclusion: Upadacitinib demonstrated sustained effectiveness and acceptable tolerability in patients with rheumatoid arthritis who had previously failed tumor necrosis factor inhibitor therapy in routine clinical practice.

Keywords: Janus kinase inhibitors, real-world evidence, rheumatoid arthritis, TNF inhibitor failure, upadacitinib.

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INTRODUCTION

Rheumatoid arthritis (RA) is a long-standing immune-mediated inflammatory condition characterized by persistent joint inflammation, progressive structural damage, and loss of

physical function, affecting an estimated 0.5–1% of the global population.[1] National epidemiological data from Türkiye similarly indicate a prevalence of 0.41–0.56%, with a gradual increase reported over the past decade.[2] This pattern has been linked to better recognition of the disease, wider availability of specialist care, and demographic shifts in the adult population.

Therapeutic approaches to RA have progressed considerably with the development of tumor necrosis factor inhibitors (TNFi), a major class of biologic disease-modifying antirheumatic drugs (bDMARDs). However, roughly 30–40% of patients either do not achieve an adequate response or experience diminished effectiveness over time, underscoring the continued need for additional therapeutic approaches.[3,4] The clinical expression of RA varies widely and is shaped by genetic, environmental, and immunologic influences. These differences lead to diverse treatment responses across populations, including those living in Türkiye.

The advent of targeted synthetic DMARDs (tsDMARDs) has expanded RA treatment options because these agents, including Janus kinase (JAK) inhibitors, offer effective oral therapy by modulating multiple cytokine signaling pathways. Upadacitinib, a selective JAK1 inhibitor, has shown consistent efficacy in multiple phase 3 randomized controlled trials (RCTs), including SELECT-BEYOND and SELECT-COMPARE, particularly in patients with an inadequate response to bDMARDs.[5,6] In RA, randomized controlled trials remain the primary standard for evaluating the efficacy and safety of emerging therapies, while complementary evidence from routine clinical practice helps place these findings in the context of broader and more heterogeneous patient populations. Observational real-world studies conducted in Germany, Italy, and Japan have reported that patients receiving upadacitinib in routine care experience significant improvements in disease activity and functional status.[7–10] However, treatment outcomes observed in routine practice may differ between regions because of variations in healthcare infrastructure, reimbursement frameworks, and population characteristics, underscoring the value of country-specific evidence.

In Türkiye, the use of upadacitinib has expanded in recent years, facilitated by comprehensive national health insurance coverage and relatively streamlined access to rheumatology services. Nevertheless, delays in diagnosis, advanced disease at presentation, socioeconomic disparities, and variable adherence patterns may influence treatment outcomes, reinforcing the need for localized real-world assessments of JAK1 inhibition in RA.

This retrospective multicenter analysis evaluated the 12-month effectiveness and safety of upadacitinib in Turkish patients with RA who had an inadequate response to at least one prior TNFi. The study drew on longitudinal data from two geographically distinct tertiary rheumatology centers, capturing routine care within the Turkish healthcare system. By evaluating these outcomes, the study aimed to generate clinically relevant evidence on the role of selective JAK1 inhibition in patients with treatment-refractory RA.

KEY MESSAGES

- Upadacitinib provided significant and sustained improvements in disease activity, functional outcomes, and patient-reported measures over 12 months in patients with RA and prior TNF inhibitor failure.
- Nearly half of the patients achieved remission or low disease activity, and no serious adverse events were recorded, supporting a favorable safety profile.
- This multicenter study adds real-world evidence from Türkiye, demonstrating that the effectiveness observed in phase 3 trials is maintained in routine clinical practice.

METHODS

This study was designed as a retrospective observational cohort and included patients treated at two tertiary rheumatology centers in Türkiye. Ethical approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (November 26, 2024). Subsequent amendments to the study protocol were reviewed and approved by the same committee at its meeting on November 4, 2025 (Meeting No. 20; official decision dated November 10, 2025, No. E-60116787-020-776848). Given that the analysis used only de-identified data obtained from routine clinical practice, individual informed consent was not required.

Eligible patients were adults aged 18 years or older with RA defined according to the 2010 ACR/EULAR classification criteria who commenced upadacitinib at a dose of 15 mg once daily after an insufficient response to at least one TNF inhibitor. The analysis used retrospectively collected data from a multicenter observational setting between October 2023 and November 2024, with information extracted from electronic medical records at baseline and at the 3-, 6-, and 12-month assessments.

Patient identification was performed through systematic screening of electronic medical records at each participating center. All consecutive patients who initiated upadacitinib during the study period were screened for eligibility according to the predefined inclusion and exclusion criteria. The final study cohort consisted of 40 patients, including 20 patients

Table 1. Baseline demographic and clinical characteristics of the study population (n=40)

Variable	Value
Mean age	48.4±11.6
Female patients	25 (62.5%)
Mean BMI	27.6±4.2
Mean disease duration	12.9±6.8
Nonsmokers	21 (52.5%)
RF positivity	23 (57.5%)
Anti-CCP positivity	22 (55.0%)
Mean number of comorbidities	3.3±1.8
Upadacitinib monotherapy	17 (42.5%)
Upadacitinib + DMARD combination therapy	23 (57.5%)

Data are presented as mean±standard deviation or n (%). BMI: Body mass index; RF: Rheumatoid factor; anti-CCP: Anti-cyclic citrullinated peptide antibody; DMARD: Disease-modifying antirheumatic drug.

from each participating center. This distribution was not based on intentional balancing or matching between centers but rather reflected the number of eligible patients with complete longitudinal clinical and laboratory follow-up data available during the study period. Only patients with complete baseline and 3-, 6-, and 12-month clinical and laboratory assessments were included in the final analysis.

Patients were excluded if they had a history of malignancy, active or chronic infections such as hepatitis B, hepatitis C, or tuberculosis, or if upadacitinib was discontinued for nonmedical reasons before the 3-month follow-up assessment. To ensure consistency in the longitudinal repeated-measures analyses, only patients with complete baseline and follow-up data through month 12 were included in the final analysis.

Baseline data collection included demographic variables, body mass index (BMI), smoking status, disease duration, comorbid conditions, serologic status for rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) antibodies, and prior exposure to conventional synthetic or biologic DMARDs. Information on concomitant treatments, including methotrexate, leflunomide, hydroxychloroquine, and systemic corticosteroids, was also recorded (Table 1).

Treatment response was evaluated according to the American College of Rheumatology (ACR) 20%, 50%, and 70% improvement criteria, incorporating proportional changes in joint counts, patient-reported outcomes, global assessments, functional measures, and inflammatory markers (Table 2).

Disease activity was assessed using the Simplified Disease Activity Index (SDAI), Clinical Disease Activity Index (CDAI),

Table 2. ACR response rates at 3, 6, and 12 months

Time point	ACR20, n (%)	ACR50, n (%)	ACR70, n (%)
3 months	27 (67.5)	14 (35.0)	3 (7.5)
6 months	32 (80.0)	28 (70.0)	8 (20.0)
12 months	35 (87.5)	31 (77.5)	16 (40.0)

Data are presented as n (%). ACR: American College of Rheumatology.

and Disease Activity Score in 28 joints using C-reactive protein (DAS28-CRP), with remission defined as DAS28-CRP <2.6 and low disease activity as DAS28-CRP <3.2. Functional status was evaluated using the Health Assessment Questionnaire (HAQ). Each visit included documentation of 28-joint tender and swollen joint counts, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values, pain assessed using a visual analog scale (VAS), and patient and physician global assessments (PtGA and PhGA) (Table 3).

Remission and low disease activity according to the SDAI and CDAI were determined using established cutoff thresholds (Supplementary Table 1).

Safety outcomes included all adverse events recorded during routine clinical follow-up. Adverse events were graded as mild, moderate, or severe based on clinical significance, including the need for hospitalization, treatment discontinuation, or additional medical intervention.

Statistical Analysis

Statistical analyses were conducted using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test, with continuous variables reported as mean±SD or median (IQR) and categorical variables as counts and percentages. Descriptive summaries in all tables and analyses followed these distribution-based reporting conventions.

Changes over time in continuous clinical and laboratory measures across the four predefined assessments were examined using the Friedman test for repeated measures. If a significant overall difference was observed, post hoc pairwise analyses were performed using the Wilcoxon signed-rank test, with Bonferroni correction applied to determine which time points accounted for the observed changes.

Changes in categorical response outcomes over time, including ACR20, ACR50, and ACR70 rates, were examined using Cochran's Q test. Pairwise post hoc comparisons were subsequently conducted using McNemar tests with Bonferroni-adjusted significance thresholds.

Table 3. Longitudinal changes in disease activity

Variable	Baseline Median (IQR)	3 months Median (IQR)	6 months Median (IQR)	12 months Median (IQR)	Friedman Test p value
SDAI	41.5 (35.2–59.4)	21.4 (13.4–31.8)	17.7 (10.0–24.0)	11.3 (6.3–18.9)	<0.001
CDAI	29.5 (22.0–40.3)	16.0 (9.0–25.3)	10.0 (6.0–16.0)	6.0 (3.0–10.5)	<0.001
DAS28-CRP	5.13 (4.74–5.97)	4.03 (2.95–4.73)	3.34 (2.62–3.92)	2.76 (1.95–3.40)	<0.001
TJC28	9 (6–12)	4 (2–8)	3 (1–5)	2 (1–4)	<0.001
SJC28	7 (5–9)	3 (1–5)	2 (1–3)	1 (0–2)	<0.001
CRP (mg/L)	21.1 (14.0–29.0)	11.0 (5.3–19.0)	8.3 (4.6–14.5)	7.0 (4.3–10.2)	<0.001
HAQ-DI	1.60 (1.2–1.9)	1.00 (0.8–1.5)	0.85 (0.6–1.2)	0.70 (0.5–0.9)	<0.001
Pain VAS	7.5 (6.5–8.2)	4.0 (4.0–6.0)	3.0 (2.0–4.0)	2.3 (1.5–3.5)	<0.001
PtGA	8.0 (7.0–9.0)	5.0 (3.0–6.0)	3.0 (2.0–4.0)	2.0 (1.0–4.0)	<0.001
PhGA	6.5 (5.8–8.0)	3.5 (3.0–5.0)	3.0 (2.0–4.0)	1.5 (1.0–3.0)	<0.001

Data are presented as median (interquartile range). Longitudinal changes across visits were evaluated using the Friedman test, followed by post hoc Wilcoxon signed-rank analyses with Bonferroni correction. SDAI: Simplified Disease Activity Index; CDAI: Clinical Disease Activity Index; DAS28-CRP: Disease Activity Score in 28 joints using C-reactive protein.

Categorical outcomes were compared using the chi-square test or Fisher's exact test, depending on the expected cell counts. All statistical analyses were two-sided, with a significance threshold of $p<0.05$.

RESULTS

The cohort comprised 40 patients with RA and a prior inadequate response to TNFi (Table 1), with a mean age of 48.4±11.6 years, a mean disease duration of 12.9±6.8 years, and 62.5% being female; the mean comorbidity count was 3.3±1.8, and the mean BMI was 27.6±4.2 kg/m^2. Seropositivity for RF and anti-CCP antibodies was observed in 57.5% and 55.0% of patients, respectively. Upadacitinib was administered as monotherapy in 42.5% of cases and in combination with conventional synthetic DMARDs in 57.5%.

ACR20/50/70 response rates at 3, 6, and 12 months are presented in Table 2 and Figure 1. At the 3-month assessment, 67.5% of patients achieved an ACR20 response, 35.0% achieved an ACR50 response, and 7.5% achieved an ACR70 response. Cochran's Q test demonstrated a significant overall change over time for all three ACR response levels, with a global p value of <0.001. At month 6, ACR20/50/70 response rates increased to 80.0%, 70.0%, and 20.0%, respectively, and further increased to 87.5%, 77.5%, and 40.0% at month 12.

Changes in disease activity measures and patient-reported outcomes over time, from baseline to the 3-, 6-, and 12-month assessments, are presented in Table 3. Friedman testing demonstrated significant improvements over time in the SDAI, CDAI, DAS28-CRP, tender and swollen joint counts, CRP, patient and physician global assessments, and pain VAS, with overall p values of <0.001 for all measures. Post hoc Wilcoxon signed-rank analyses with Bonferroni adjustment confirmed statistically significant reductions at each follow-up visit compared with baseline.

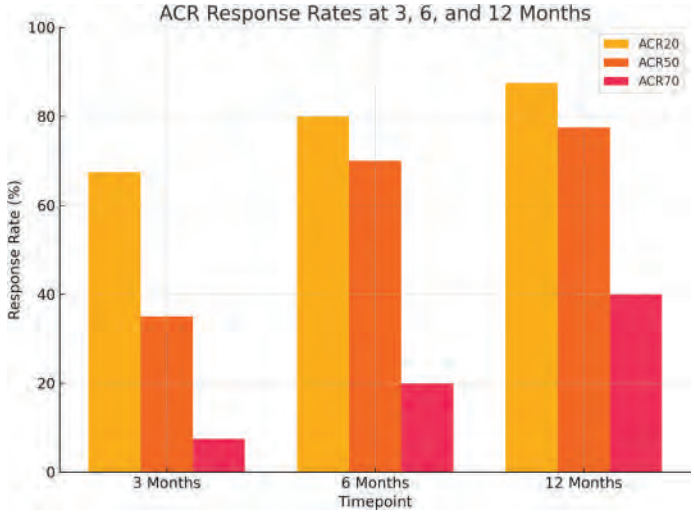

Figure 1. ACR20/50/70 response rates at 3, 6, and 12 months. Bars represent the proportion of patients achieving ACR20, ACR50, and ACR70 responses at each follow up visit.

Functional outcomes improved steadily over the 12-month follow-up. HAQ-DI scores decreased progressively over the 12-month follow-up, indicating improvement in physical function. At the 12-month assessment, 45.0% of patients achieved low disease activity or remission according to the SDAI, while 70.0% achieved the corresponding target according to the CDAI. Rates of remission and low disease

activity defined by the SDAI, CDAI, and DAS28-CRP at each follow-up visit are summarized in Supplementary Table 1.

No serious adverse events occurred during the 12-month follow-up; mild upper respiratory tract infections were reported in four patients (10.0%). Three patients (7.5%) experienced temporary, asymptomatic increases in alanine/aspartate aminotransferase levels to less than two times the upper limit of normal, which resolved without treatment discontinuation. There were no reports of herpes zoster (HZ), venous thromboembolism (VTE), major adverse cardiovascular events (MACE), malignancy, or death.

DISCUSSION

This multicenter retrospective cohort study evaluated the 12-month outcomes of upadacitinib in patients with RA who had a prior inadequate response to TNFi therapy.[11] Improvements across disease activity indices, patient-reported assessments, and functional status indicate that the benefits observed in phase 3 trials are maintained in routine clinical care.[12] The magnitude and trajectory of the clinical response in this cohort underscore the therapeutic value of selective JAK1 inhibition in a population commonly characterized by prolonged disease duration and complex treatment histories.[13]

When the results from our cohort are viewed alongside those of major randomized controlled trials, such as SELECT-BEYOND and SELECT-COMPARE, the early gains in ACR20 responses appear generally comparable to those reported in these studies.[14] While RCTs continue to represent the standard for evaluating efficacy and safety, studies conducted in routine clinical settings provide complementary evidence by capturing outcomes in more diverse and heterogeneous patient populations.[15] The relatively modest ACR50 and ACR70 rates observed at early follow-up visits likely reflect the realities of daily clinical practice, including a higher burden of comorbidities, longer disease duration, and the inclusion of patients who had previously failed multiple biologic therapies.[16] These characteristics are well-recognized factors associated with lower rates of rapid and deep treatment responses in RA.[17] Despite these unfavorable baseline characteristics, ACR20/50/70 response rates increased progressively over the 12-month follow-up, indicating that upadacitinib retains clinically meaningful effectiveness even in treatment-refractory settings.[18] This pattern is consistent with findings from international real-world cohorts reported from Germany, Italy, Japan, and Canada, as well as with systematic evaluations of JAK inhibitor effectiveness in routine practice.[19]

Upadacitinib treatment was associated with sustained improvements across several disease activity measures, including the SDAI, CDAI, and DAS28-CRP, reflecting a broad therapeutic effect accompanied by parallel reductions in inflammatory markers and clinical joint findings.[11] Improvements in patient-reported outcomes, such as pain and global assessment scores, mirrored these objective changes. Functional outcomes also improved consistently over time, supporting the clinical relevance of the observed reductions in disease activity. Collectively, these improvements across multiple domains resulted in steadily increasing rates of remission and low disease activity, aligning with treat-to-target strategies in RA management. The overall clinical trajectory observed in our cohort closely aligns with real-world findings from multiple European and international settings and complements 1-year outcomes reported in both observational cohorts and extension phases of randomized trial programs evaluating upadacitinib.

With respect to safety, upadacitinib was generally well tolerated throughout the 12-month observation period. No serious adverse events were recorded, and only mild infections and transient elevations in liver transaminase levels were observed. Given the modest sample size, the absence of adverse events of special interest, such as HZ, VTE, MACE, or malignancy, should be interpreted cautiously. Nevertheless, the observed safety profile is broadly consistent with data reported from long-term extension studies and large real-world registries evaluating JAK inhibitors.

An important strength of this study is that it reflects routine clinical practice within the Turkish public healthcare system. Because the participating centers are located in different geographic regions of Türkiye yet operate under a unified national reimbursement and treatment framework, the findings are likely to be representative of everyday rheumatology care at the national level. This study contributes valuable country-specific real-world evidence and helps address a current gap in regional data regarding the use of targeted synthetic disease-modifying antirheumatic drugs.

Several limitations warrant consideration, as the retrospective study design is inherently subject to potential information and selection biases. The absence of a comparator group limits direct comparisons with other biologic or targeted synthetic therapies, and the modest sample size constrains detailed subgroup analyses and the evaluation of infrequent safety events. Additionally, restricting the analysis to patients with complete 12-month follow-up may have introduced selection bias by excluding patients who discontinued treatment or were lost to follow-up earlier. Despite these limitations, the consistency of the findings across validated disease activity measures and the robustness of the repeated-measures analyses support the overall reliability of the observed results. Further clarification of long-term safety and optimal treatment sequencing will require larger prospective and comparative real-world studies with extended follow-up.

CONCLUSION

Across 12 months of follow-up, upadacitinib demonstrated sustained clinical effectiveness and was generally well tolerated in Turkish patients with RA who had an inadequate response to TNFi therapy. These findings add to the real-world evidence supporting upadacitinib in difficult-to-treat RA and are broadly consistent with outcomes reported in controlled clinical trials.

Ethics Committee Approval: Ethical approval was obtained from the Pamukkale University Non-Interventional Clinical Research Ethics Committee (November 26, 2024). Subsequent amendments to the study protocol were reviewed and approved by the same committee at its meeting on November 4, 2025 (Meeting No. 20; official decision dated November 10, 2025, No. E-60116787-020-776848).

Informed Consent: Due to the retrospective nature of the study and anonymized data, the requirement for informed consent was waived.

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Supplementary Table 1. Remission and low disease activity rates

Index	3-month remission, n (%)	3-month LDA/ remission, n (%)	6-month remission, n (%)	6-month LDA/ remission, n (%)	12-month remission, n (%)	12-month LDA/ remission, n (%)
DAS28-CRP	5 (12.5)	13 (32.5)	9 (22.5)	20 (50.0)	17 (42.5)	28 (70.0)
SDAI	0 (0.0)	4 (10.0)	0 (0.0)	13 (32.5)	4 (10.0)	18 (45.0)
CDAI	1 (2.5)	11 (27.5)	1 (2.5)	19 (47.5)	6 (15.0)	28 (70.0)

Data are presented as n (%). Remission and low disease activity were defined according to the established cutoff values for each index. LDA: low disease activity; DAS28-CRP: Disease Activity Score in 28 joints using C-reactive protein; SDAI: Simplified Disease Activity Index; CDAI: Clinical Disease Activity Index.

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Does the Initial Source of Recognition—Clinician versus Caregiver—Affect the Age at Orchidopexy for Undescended Testis? A Retrospective Comparative Analysis of 283 Cases

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ABSTRACT

Objective: To evaluate institutional adherence to international guidelines regarding the timing of treatment for undescended testis (UDT) and investigate the impact of the initial source of recognition on age at orchidopexy.

Materials and Methods: We conducted a retrospective analysis of 283 patients who underwent orchidopexy for UDT between 2012 and 2022. Chronological age, laterality, and the initial source of recognition (clinician vs. primary caregiver) were evaluated using IBM SPSS Statistics, version 28. Variables were compared using the chi-square test, independent-samples t-test, or Mann–Whitney U test, with statistical significance defined as $p<0.05$.

Results: The mean age at orchidopexy was 4.8±5.6 years, reflecting an overall trend toward delayed consultation. A subgroup analysis of 185 patients with documented recognition data showed that caregiver-detected cases underwent surgery at a significantly older age than clinician-detected cases (5.08±4.98 vs. 3.57±3.68 years; $p=0.020$). A sensitivity analysis excluding adult patients confirmed that this difference remained significant within the pediatric cohort ($p=0.016$). However, most procedures in both groups were performed well beyond the recommended 18-month guideline threshold.

Conclusion: Delayed orchidopexy remains a prevalent clinical challenge with potential long-term implications for testicular health. Although clinician-led detection significantly improves surgical timing compared with caregiver recognition, systemic improvements in early primary care screening and caregiver awareness are essential to ensure strict adherence to optimal surgical timelines.

Keywords: Cryptorchidism, infertility, orchidopexy, primary care, testicular cancer, undescended testicle.

INTRODUCTION

Cryptorchidism, or undescended testis (UDT), is the most common congenital malformation affecting the male reproductive system, occurring in up to 4.6% of full-term infants and up to 45% of preterm newborns.[1] Although UDT is typically congenital, acquired forms characterized by the secondary ascent of a previously descended testis—referred to as ascending testis—may also occur. In congenital cases, spontaneous descent into the scrotum may occur within the first 6 months of life; however, spontaneous resolution after this period is exceedingly rare.[2]

The clinical significance of UDT lies in its potential to cause serious long-term complications, including impaired fertility, testicular torsion, and malignant transformation. Histopathological studies indicate that delaying surgical intervention beyond the first year of life leads to progressive interstitial fibrosis and significant impairment of spermatogenesis, thereby directly contributing to an increased risk of subsequent male infertility.[3–5] Furthermore, delayed surgery is strongly associated with an increased risk of testicular germ cell tumors, with the risk increasing substantially when orchidopexy is deferred until after adolescence.[6] Recent epidemiological data suggest that every 6-month delay in testicular descent into the scrotum is associated with a 6% incremental increase in the risk of testicular malignancy.[7]

Adherence to evidence-based treatment guidelines is essential to mitigate these risks. The American Urological Association (AUA) and European Association of Urology (EAU) guidelines recommend that, if spontaneous descent does not occur by 6 months of age, surgical orchidopexy should ideally be performed within the following year and completed no later than 18 months of age to preserve reproductive potential and facilitate oncological surveillance.[8,9]

Despite these well-established clinical consensus statements, timely surgical intervention remains a global challenge. Delays often result from systemic gaps in early detection, screening protocols, and referral pathways. This study aimed to evaluate adherence to international guidelines for UDT management at our tertiary institution. Specifically, we investigated the chronological age at orchidopexy and evaluated whether the initial source of clinical recognition influenced the timing of definitive surgery.

KEY MESSAGES

- Timely detection of undescended testis (UDT) is vital because delayed treatment remains a major risk factor for future male infertility and testicular malignancy.
- Although clinician-detected cases are diagnosed earlier than caregiver-identified cases, both groups frequently miss the 18-month "gold-standard" window for orchidopexy.
- Addressing this delay requires a dual strategy: implementing more rigorous professional screening protocols during infancy and enhancing caregiver education to promote prompt recognition and specialist referral.

MATERIALS AND METHODS

Study Protocol and Design

This retrospective descriptive study was conducted in the urology and pediatric surgery clinics of our tertiary academic center. The study protocol adhered to the ethical principles of the Declaration of Helsinki and was approved by Ordu University Clinical Research Ethics Committee (Approval Number: 268, Date: 25.11.2022).

Patients and Data Collection

We reviewed the medical records of patients diagnosed with UDT who underwent orchidopexy in our urology and pediatric surgery departments between January 1, 2012, and October 15, 2022. A total of 283 patients met the eligibility criteria. The patient selection process and study flow are summarized in Figure 1.

Clinical data, including chronological age at orchidopexy and UDT laterality, were systematically extracted from institutional electronic health records. We also documented the initial source of recognition, categorized as clinician-identified (detected during routine medical examinations or well-baby visits) or caregiver-identified (recognized by parents or guardians), along with the dates of surgical intervention.

Inclusion and Exclusion Criteria

The study included only patients with palpable UDT located in the proximal groin, distal groin, or proximal scrotum who underwent definitive orchidopexy. We excluded patients with:

- Incomplete clinical notes or missing essential surgical records.
- Documented spontaneous descent before the scheduled surgery.
- Nonpalpable testes, including intra-abdominal or vanishing testes.
- Retractile testes.
- Additional complex congenital anomalies requiring concomitant major surgical procedures.

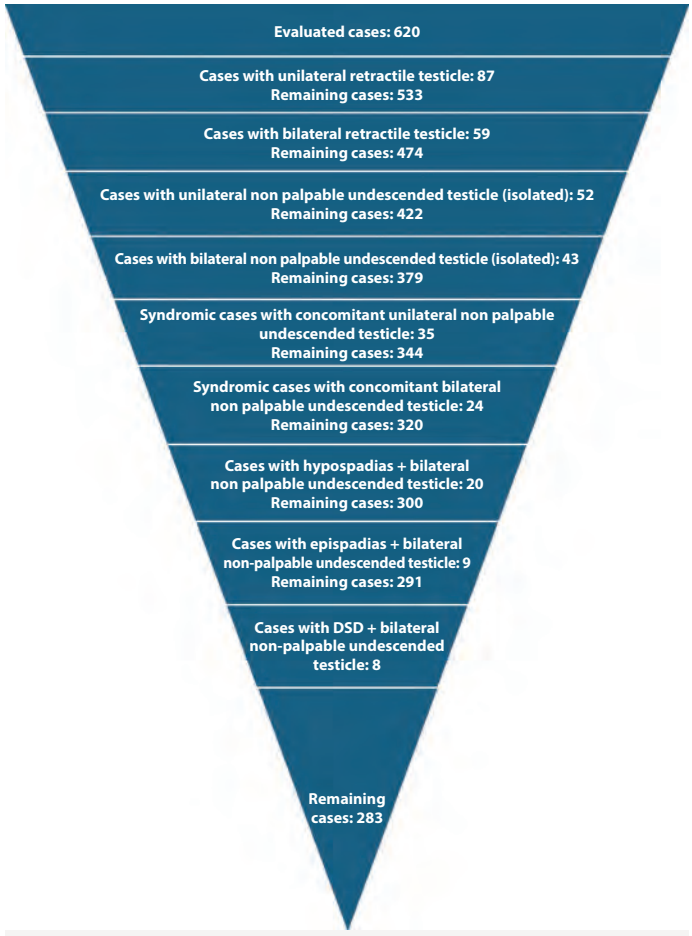

Figure 1. Flowchart of patient selection for the study.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0 (IBM Corp., Armonk, NY, USA). The distributions of continuous variables were assessed using visual methods, including histograms and probability plots, and analytical tests, including the Kolmogorov–Smirnov and Shapiro–Wilk tests. Normally distributed data are expressed as mean±standard deviation (SD), whereas nonnormally distributed variables are reported as median (interquartile range [IQR]). Categorical variables are summarized as numbers and percentages.

For comparisons of continuous variables between groups, the independent-samples t-test was used for normally distributed data, whereas the Mann–Whitney U test was used for nonparametric data. Associations between categorical variables were evaluated using the chi-square test. Pairwise deletion was used to address missing retrospective data in subgroup analyses. A p value of <0.05 was considered statistically significant.

Table 1. Distribution of the initial source of recognition in the study population (N=283)

Recognition source	Count (n)	Percentage (%)
Caregiver-identified	92	32.5
Clinician-identified	93	32.9
Undocumented	98	34.6
Total	283	100.0

Table 2. Subgroup analysis comparing age at orchidopexy according to the source of recognition (n=185)*

Recognition source	n	Mean age at surgery (years)±SD	p
Clinician-identified	93	3.57±3.68	0.020
Caregiver-identified	92	5.08±4.98	

*: Excludes 98 patients with undocumented recognition sources. Statistical analysis was performed using the independent-samples t-test.

RESULTS

General Characteristics of the Study Population

A total of 283 male patients who underwent orchidopexy for UDT were included in the baseline analysis. Regarding laterality, UDT was left-sided in 31.4% (n=89) of patients, right-sided in 21.9% (n=62), and bilateral in 11.3% (n=32). In 35.3% (n=100) of cases, laterality was not documented in the historical records.

The baseline distribution of the initial source of recognition is summarized in Table 1. The condition was first identified by primary caregivers (parents) in 32.5% (n=92) of cases and by clinicians during medical assessments in 32.9% (n=93). In the remaining 34.6% (n=98) of patients, the initial source of recognition was not documented.

Surgical Timing and Clinical Correlates

The overall mean age at orchidopexy in the entire cohort was 4.80±5.66 years (range, 0–45 years; median, 3.0 years). The mean age at surgery did not differ significantly according to UDT laterality (left, right, or bilateral; p=0.390, one-way analysis of variance).

Comparative analyses based on the initial source of recognition were restricted to the 185 patients with complete data, whereas the remaining 98 patients with undocumented recognition sources were excluded from this subgroup analysis. As detailed in Table 2, a significant association was observed between the initial source of recognition and age at surgical intervention (p=0.020). The mean age at orchidopexy was significantly lower in the clinician-detected group than in the caregiver-detected group (3.57±3.68 years vs. 5.08±4.98 years, respectively; p=0.020).

Pediatric Sensitivity Analysis

Because our cohort included a small number of adult patients with late historical presentations, including patients up to 45 years of age, a sensitivity analysis was performed by restricting the cohort to pediatric patients aged <18 years (n=277) to confirm the validity of our findings. In this pediatric-only group, the mean age at orchidopexy was 3.24±2.85 years in the clinician-identified subgroup (n=91), compared with 4.62±3.41 years in the caregiver-identified subgroup (n=90). The difference remained statistically significant (t=−2.43, p=0.016; independent-samples t-test), confirming that clinician detection was consistently associated with earlier surgical correction.

Despite this statistically significant difference, the mean age in both groups substantially exceeded the ideal 18-month threshold recommended by international guidelines.

DISCUSSION

The primary clinical objective of undescended testis management is the timely surgical relocation of the gonad into the scrotum. In the existing literature, the rationale for early orchidopexy rests on two main principles: optimizing long-term fertility and reducing the risk of testicular malignancy or facilitating its surveillance. Histopathological evidence has clearly established that structural changes within the cryptorchid gonad, such as interstitial fibrosis and germ cell loss, progress with age and may become irreversible after the first year of life.[10]

Despite the consensus among major international guidelines, including those of the AUA and EAU, that surgical intervention should be completed between 6 and 18 months of age, global data consistently demonstrate a persistent gap between guideline recommendations and clinical practice. Large-scale reports indicate that approximately 58%–83% of patients with UDT worldwide undergo orchidopexy after 18 months of age, with median ages at surgery ranging widely from 23 to 64 months.[11–20] Even after the widespread dissemination of the 2014 AUA guidelines, recent multi-institutional studies have shown that systemic surgical delays remain largely unresolved.[21]

A key finding of our study was that the age at orchidopexy was significantly lower when the condition was first identified by a clinician during a routine medical assessment rather than by a primary caregiver (3.57 years vs. 5.08 years; p=0.020). This difference was further confirmed by our sensitivity analysis excluding adult outliers (p=0.016), emphasizing that physical examinations performed by trained healthcare professionals may expedite definitive surgical care. Caregivers often lack the anatomical knowledge required to distinguish a truly undescended testis from a retractile testis or normal physiological variation, which may result in delayed consultation until the child approaches school age or undergoes a routine preschool evaluation.

However, a particularly concerning finding in our dataset was that, even in the clinician-detected group, the mean age at surgery (3.57±3.68 years) remained substantially higher than the recommended 18-month threshold. This finding indicates that clinician recognition alone, in its current form, is insufficient. Gaps may exist not only in caregiver awareness but also in the frequency of routine penoscrotal examinations during infant well-baby visits, the timeliness of specialist referrals by primary care practitioners, and the misclassification of true UDT as a harmless retractile testis.

Multifaceted strategies are therefore urgently needed to address this clinical gap. These strategies should include reinforcing mandatory genital examinations during routine immunization visits, implementing electronic health record reminders for primary care providers, and distributing locally adapted educational brochures to caregivers. Furthermore, as prospective public health models suggest, future research should evaluate whether delays in caregiver recognition are driven solely by educational deficiencies or are compounded by systemic socioeconomic barriers, health insurance disparities, and geographic limitations in access to specialized tertiary care.[22,23]

Strengths and Limitations

The primary strength of this study is its multidisciplinary approach, which combined surgical data from both the urology and pediatric surgery clinics of an academic institution, thereby reducing potential bias associated with individual practitioners.

However, several limitations must be acknowledged. First, the retrospective design carries inherent risks of incomplete documentation. The substantial proportions of missing data regarding UDT laterality (35.3%) and the initial source of recognition (34.6%) represent major limitations and may have introduced selection or information bias. Second, our database did not allow us to reliably distinguish historical cases of ascending testis, or acquired cryptorchidism, from true congenital cases with delayed presentation, which may have artificially increased the calculated mean age at surgery. Finally, the lack of standardized electronic documentation regarding patients' socioeconomic status, insurance type, and distance from our center precluded the development of a multivariable model to account for potential confounding factors.

CONCLUSION

Undescended testis is a time-sensitive condition requiring surgical correction within a strict therapeutic window to protect future reproductive and oncological health. Significant delays in orchidopexy persist both globally and regionally. Our study demonstrates that, although clinician-led detection significantly reduces the age at surgery compared with caregiver recognition, the timing of surgery in both groups remains delayed relative to established international guidelines.

To achieve optimal surgical timing, targeted educational initiatives for primary care physicians and family practitioners are essential. Primary care providers should prioritize standardized penoscrotal examinations during all routine infant visits and maintain a low threshold for early surgical referral, recognizing that true congenital undescended testes are unlikely to descend spontaneously after the neonatal period.

Ethics Committee Approval: Ethics committee approval was obtained from Ordu University Clinical Research Ethics Committee (Approval Number: 268, Date: 25.11.2022).

Informed Consent: Informed consent was not required due to the retrospective nature of this study.

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Endothelial Biomarkers Predict Clinical Deterioration in Acute Pancreatitis

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ABSTRACT

Objective: Endothelial dysfunction contributes to the severity of acute pancreatitis (AP), but the prognostic utility of endothelial biomarkers and their association with therapeutic interventions remain poorly defined. This study aimed to determine whether endothelial dysfunction biomarkers predict clinical deterioration in AP and whether plasmapheresis is associated with changes in these biomarkers and clinical outcomes.

Materials and Methods: In this prospective cohort study of 100 patients with AP, serial endothelial biomarkers, including vascular endothelial growth factor (VEGF), von Willebrand factor (vWF), endothelin-1 (ET-1), and soluble E-selectin (sE-selectin), were measured on admission and on days 3 and 7. All patients received conventional therapy, and 68 additionally received adjunctive plasmapheresis through nonrandomized assignment. The conventional therapy cohort (n=32) was stratified into Worsened (n=17) and Improved (n=15) subgroups according to clinical course to establish prognostic biomarker cutoffs.

Results: Endothelial marker levels at admission predicted subsequent clinical deterioration ($p<0.001$). Biomarker trajectories differed significantly between the Improved and Worsened subgroups. Patients who received plasmapheresis exhibited more favorable biomarker profiles, with levels resembling those of the Improved subgroup by day 7. This biomarker pattern was associated with a lower rate of the composite clinical deterioration endpoint than that observed in the Worsened subgroup (12% vs. 100%, $p<0.001$).

Conclusion: Endothelial dysfunction biomarkers are early predictors of the clinical course of AP. Plasmapheresis was associated with favorable biomarker trajectories and improved clinical outcomes, suggesting that endothelial dysfunction may be a potential therapeutic target warranting investigation in randomized trials.

Keywords: Acute pancreatitis, biomarkers, endothelial dysfunction, plasmapheresis, prognosis.

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease with a highly variable clinical course, ranging from a mild, self-limited illness to severe disease characterized by persistent organ failure, multiorgan dysfunction, and high mortality.[1] Early identification of patients at risk of clinical deterioration

remains a major challenge because currently available scoring systems have limited predictive accuracy within the first 48–72 hours.[2] Accumulating evidence suggests that endothelial dysfunction may be a central pathophysiological mechanism linking early pancreatic injury to systemic inflammation and organ failure in AP.[3]

Endothelial injury in critical illness is characterized by glycocalyx degradation, increased vascular permeability, and dysregulation of hemostatic pathways.[4] Key biomarkers include von Willebrand factor (vWF), angiopoietin-2, and glycocalyx components.[5] Similar alterations occur in septic shock, inflammatory bowel disease, and hepatic malignancies, in which elevated vWF levels correlate with disease severity.[6–8] Comparable endothelial changes are also thought to occur in severe AP; however, this area remains insufficiently explored.[9]

Recent studies of septic shock—a condition that shares systemic inflammation and microvascular failure with severe AP—have shown that endothelial biomarkers are closely associated with disease severity and may reflect underlying pathophysiological processes.[10] In this context, therapeutic plasma exchange (TPE) has been associated with reductions in circulating mediators and improvements in endothelial markers.[10,11] Although TPE is an established therapy for hypertriglyceridemic pancreatitis, its use in this setting has primarily been directed toward reducing the triglyceride burden.[1] Whether TPE can influence endothelial dysfunction in AP independently of triglyceride reduction remains an important unanswered question. Given the shared pathways of cytokine release, oxidative stress, and microvascular injury in sepsis and severe AP, endothelial dysfunction biomarkers may serve as promising early prognostic indicators.[12] However, two major knowledge gaps remain: (1) the prognostic utility of endothelial dysfunction biomarkers in AP has not been established, and (2) the association between plasmapheresis and changes in the trajectories of these biomarkers in AP is unknown.

The present study aimed to (1) evaluate the ability of endothelial dysfunction biomarkers to predict clinical deterioration in AP and (2) assess whether plasmapheresis is associated with changes in these biomarkers and clinical outcomes.

MATERIALS AND METHODS

Study Setting and Design

This prospective observational cohort study was conducted between 2019 and 2022 at a tertiary surgical center. The study was designed to evaluate the prognostic utility of endothelial dysfunction biomarkers and assess the association between plasmapheresis and biomarker trajectories in patients with acute pancreatitis.

KEY MESSAGES

- Serial endothelial biomarkers—particularly sE-selectin and endothelin-1—strongly predict clinical deterioration in acute pancreatitis, with sE-selectin levels on day 3 demonstrating excellent predictive accuracy (AUC=0.905).
- Plasmapheresis was associated with favorable biomarker trajectories and a significantly lower rate of clinical deterioration (12% vs. 100% in the Worsened subgroup, p<0.001).
- Endothelial dysfunction may represent a modifiable therapeutic target in acute pancreatitis, supporting the need for randomized trials of biomarker-guided interventions.

Ethics Approval

The study protocol was approved by the Academic M.A. Topchubashov Scientific Surgery Center Ethics Committee (Approval Number: 6, Date: 17.09.2019). Written informed consent was obtained from all participants before enrollment. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Patients and Data Collection

A total of 100 patients with acute pancreatitis were consecutively enrolled. The collected data included demographic characteristics, clinical parameters, laboratory measurements, and clinical outcomes. Peripheral blood samples were collected on days 1, 3, and 7 of hospitalization. Plasma was separated and stored at −80°C for subsequent biomarker analysis.

Diagnostic Criteria

The diagnosis of acute pancreatitis was established according to the Revised Atlanta Classification and required at least two of the following three criteria: (1) abdominal pain consistent with acute pancreatitis; (2) serum amylase and/or lipase levels ≥3 times the upper limit of normal; and (3) characteristic findings on contrast-enhanced computed tomography, magnetic resonance imaging, or ultrasonography.

Etiology of Acute Pancreatitis

Biliary stone disease was the predominant etiological factor and was identified in 83 patients (83%). A pancreatic cyst was detected in 6 patients (6%). In 11 patients (11%), no definitive etiological factor could be identified despite a comprehensive diagnostic workup; therefore, these cases were classified as idiopathic acute pancreatitis. No cases of hypertriglyceridemia-

induced or alcohol-induced pancreatitis were recorded in this cohort. The distribution of etiologies was similar between the plasmapheresis and conventional therapy groups (p=0.79).

Definitions

Clinical deterioration: A composite endpoint defined as in-hospital mortality or new or worsening organ failure requiring admission to the intensive care unit (ICU). Organ failure was defined as a Modified Marshall score ≥2 for any organ system.

- Improved subgroup: Patients in the conventional therapy group who had no organ failure, experienced an uncomplicated clinical course, and did not require ICU admission.
- Worsened subgroup: Patients in the conventional therapy group who developed new or persistent organ failure (Modified Marshall score ≥2), required ICU admission, or died.

Inclusion Criteria

The inclusion criteria were as follows: (1) age ≥18 years; (2) diagnosis of acute pancreatitis according to the Revised Atlanta Classification; and (3) admission within 7 days of symptom onset.

Exclusion Criteria

The exclusion criteria were as follows: (1) chronic pancreatitis; (2) pancreatic malignancy; (3) pregnancy; (4) transfer from another hospital more than 48 hours after admission; and (5) refusal to provide informed consent.

Study Groups and Treatment Allocation

All patients received standard guideline-recommended therapy.

Standard Conventional Therapy

All patients received standard guideline-recommended therapy for acute pancreatitis, including aggressive intravenous fluid resuscitation with crystalloids (Ringer's lactate or normal saline), early enteral nutrition through a nasogastric or nasoduodenal tube when oral intake was not tolerated, and analgesia as required. Patients with organ failure received supportive care in the ICU, including vasopressor support for hemodynamic instability, mechanical ventilation for respiratory failure, and renal replacement therapy for acute kidney injury when indicated. Antibiotics were administered only in cases of documented or suspected infected pancreatic necrosis. All therapeutic decisions were made by the treating clinical team in accordance with international guidelines for the management of acute pancreatitis.

The decision to administer adjunctive plasmapheresis was made by the treating physician based on clinical judgment regarding disease severity. Treatment allocation was nonrandomized. Patients were divided into two groups:

- Plasmapheresis group (n=68): Conventional therapy plus plasmapheresis.
- Conventional therapy group (n=32): Conventional therapy alone.

For the secondary analysis, the conventional therapy group was subdivided post hoc into:

- Worsened subgroup (n=17): Patients who met the definition of clinical deterioration.
- Improved subgroup (n=15): Patients who did not experience clinical deterioration.

Plasmapheresis Procedure

Plasmapheresis was performed using a centrifugal apheresis platform (Spectra Optia, Terumo BCT, Tokyo, Japan). Each session lasted 1–3 hours and used 5% human albumin as the replacement fluid at a volume equivalent to 1.0–1.5 times the patient's estimated plasma volume. Procedures were performed 2–3 times weekly based on the patient's clinical status.

Laboratory Investigations

Biomarker assessment: Peripheral blood samples were collected on days 1, 3, and 7 of hospitalization. Plasma was separated by centrifugation and stored at −80°C until analysis. Soluble E-selectin (sE-selectin) and von Willebrand factor (vWF) were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cloud-Clone Corp., Katy, TX, USA) according to the manufacturer's protocols. Vascular endothelial growth factor (VEGF) and endothelin-1 (ET-1) were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA). All samples were analyzed in duplicate, and concentrations were determined using standard curves.

All biomarker assays were performed by laboratory personnel who were blinded to clinical outcomes and patient group allocation.

Clinical laboratory measurements: Serum amylase and lipase levels were measured at admission using standard automated laboratory methods. Organ failure was assessed daily during the first week of hospitalization using the Modified Marshall scoring system.

Statistical Analysis

Continuous variables were assessed for normality using the Shapiro–Wilk test and visual inspection of Q–Q plots. Normally distributed data were expressed as mean±standard deviation (SD) and compared using two-tailed independent-samples t tests for two-group comparisons or one-way analysis of variance (ANOVA) with post hoc tests for comparisons

Table 1. Baseline characteristics of patients with acute pancreatitis

Characteristic	All patients (n=100)	Plasmapheresis group (n=68)	Conventional therapy group (n=32)	p
Age, years	48.19±1.46	47.8±1.8	49.0±2.1	0.67
Sex, male/female	46/54	32/36	14/18	0.82
BMI, kg/m^2	28.57±0.44	28.3±0.5	29.1±0.7	0.34
BMI category, n, (%)				0.71
Underweight	1 (1)	1 (1.5)	0	
Normal weight (18.5–24.9)	17 (17)	12 (17.6)	5 (15.6)	
Overweight (25.0–29.9)	46 (46)	32 (47.1)	14 (43.8)	
Class I obesity (30.0–34.9)	32 (32)	20 (29.4)	12 (37.5)	
Class II obesity (35.0–39.9)	2 (2)	2 (2.9)	0	
Class III obesity (≥40.0)	2 (2)	1 (1.5)	1 (3.1)	
Comorbidities, n, (%)				
Diabetes mellitus	13 (13)	9 (13.2)	4 (12.5)	1.00
Hepatic steatosis	29 (29)	20 (29.4)	9 (28.1)	0.89
Arterial hypertension	12 (12)	8 (11.8)	4 (12.5)	1.00
Ischemic heart disease	9 (9)	6 (8.8)	3 (9.4)	1.00
COPD	4 (4)	3 (4.4)	1 (3.1)	1.00
Chronic kidney disease	4 (4)	3 (4.4)	1 (3.1)	1.00
Symptom duration at admission, n, (%)				0.45
≤5 days	27 (27)	20 (29.4)	7 (21.9)	
6–10 days	12 (12)	9 (13.2)	3 (9.4)	
≥11 days	61 (61)	39 (57.4)	22 (68.8)	

Data are presented as mean±standard error of the mean or number (%). p values compare the plasmapheresis and conventional therapy groups. BMI: Body mass index; COPD: Chronic obstructive pulmonary disease.

among multiple groups. Non-normally distributed data were expressed as median and interquartile range (IQR) and compared using the Mann–Whitney U test or Kruskal–Wallis test. Categorical variables were expressed as frequencies and percentages and compared using the chi-square test or Fisher's exact test, as appropriate.

Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive ability of biomarkers for clinical deterioration. Areas under the curve (AUCs) with 95% confidence intervals (CIs) were calculated, and optimal cutoff values were determined using Youden's index.

To address potential confounding resulting from nonrandomized treatment allocation, multivariable logistic regression analysis was performed to assess the independent association between plasmapheresis and clinical deterioration. The model was adjusted for baseline covariates, including age, body mass index (BMI), and admission levels of VEGF, vWF, ET-1, and sE-selectin. The results are reported as adjusted odds ratios (ORs) with 95% CIs. A two-sided $p<0.05$ was considered statistically significant.

Biomarker trajectories over time were compared among the groups using repeated-measures ANOVA, with F statistics and degrees of freedom reported. Correlations between biomarker levels and clinical outcomes were assessed using Pearson or Spearman correlation coefficients, as appropriate, with 95% CIs reported for the primary correlations.

Survival analysis was performed using the Kaplan–Meier method, with patients censored at hospital discharge or at the end of the 30-day follow-up period. Survival curves were compared using the log-rank test, and median survival times with 95% CIs were reported.

No adjustment was made for multiple comparisons because these analyses were exploratory. Missing data were minimal (<5%) and were handled using complete-case analysis.

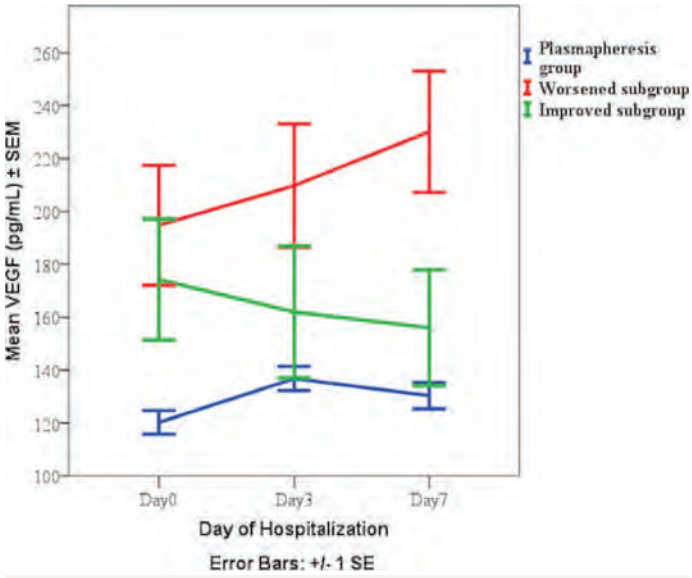

Figure 1. VEGF trajectories in patients with acute pancreatitis. Mean±SEM levels of vascular endothelial growth factor (VEGF) on days 1, 3, and 7 in the plasmapheresis group (n=68), Worsened subgroup (n=17), and Improved subgroup (n=15).

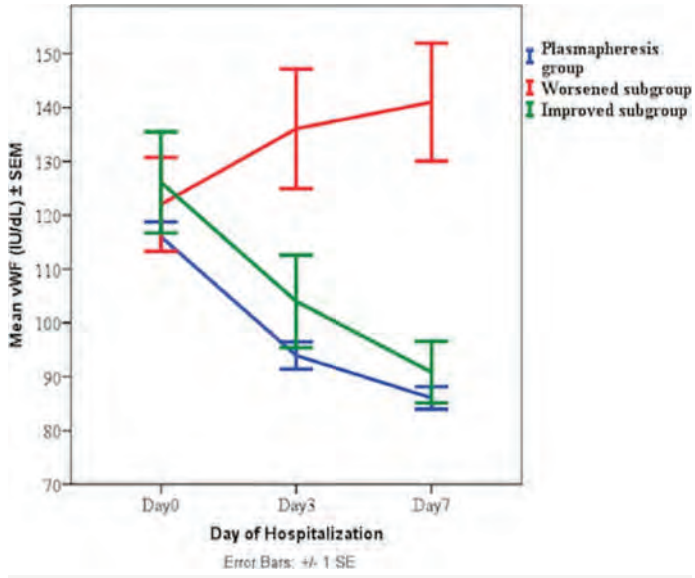

Figure 2. vWF trajectories in patients with acute pancreatitis. Mean±SEM levels of von Willebrand factor (vWF) on days 1, 3, and 7 in the plasmapheresis group (n=68), Worsened subgroup (n=17), and Improved subgroup (n=15).

Outliers were identified through boxplot inspection and retained in the analysis after verification. A two-sided p<0.05 was considered statistically significant.

All analyses were performed using IBM SPSS Statistics, version 22 (IBM Corp., Armonk, NY, USA).

RESULTS

Baseline Characteristics and Admission Biomarker Levels

Baseline characteristics are summarized in Table 1. The plasmapheresis and conventional therapy groups were comparable, although the conventional therapy group had higher admission levels of VEGF (185.14 vs. 120.20 pg/mL, p<0.05) and ET-1 (p<0.001). Within the conventional therapy group, the Worsened subgroup (n=17) had higher baseline levels of all biomarkers than the Improved subgroup (n=15), although the differences in VEGF and sE-selectin levels did not reach statistical significance.

Biomarker Trajectories

Serial biomarker measurements revealed distinct temporal patterns according to treatment group and clinical course.

VEGF: Repeated-measures ANOVA demonstrated significant differences in VEGF trajectories among the groups (F=51.585, p<0.001). The plasmapheresis group showed a progressive decline from day 1 to day 7. In contrast, the Worsened subgroup exhibited increasing VEGF levels over time, whereas the Improved subgroup showed a gradual decline (Fig. 1). Higher VEGF levels on days 3 and 7 were correlated with prolonged hospitalization and a longer ICU stay (p<0.05).

von Willebrand factor (vWF): vWF levels decreased over time in the plasmapheresis group but increased in the Worsened subgroup. By day 7, the mean vWF level was 86.04 IU/dL in the plasmapheresis group compared with 141.01 IU/dL in the Worsened subgroup (p<0.001) (Fig. 2). vWF showed a weak but significant association with the severity of organ failure.

Endothelin-1: ET-1 trajectories differed markedly among the groups throughout treatment (F=952.966, p<0.001). The plasmapheresis group demonstrated a rapid decline, whereas the Worsened subgroup maintained persistently elevated levels (Fig. 3). High ET-1 levels on days 3 and 7 were strongly associated with mortality (r≈–0.36 to –0.48, p<0.001) and prolonged hospitalization.

Soluble E-selectin (sE-selectin): sE-selectin levels declined steadily in the plasmapheresis group but remained elevated in the Worsened subgroup (p<0.001) (Fig. 4). Higher sE-selectin levels on days 3 and 7 were correlated with longer hospitalization and a lower probability of survival (p<0.001) (Fig. 5).

Multivariable Analysis

To determine whether plasmapheresis remained independently associated with clinical deterioration after

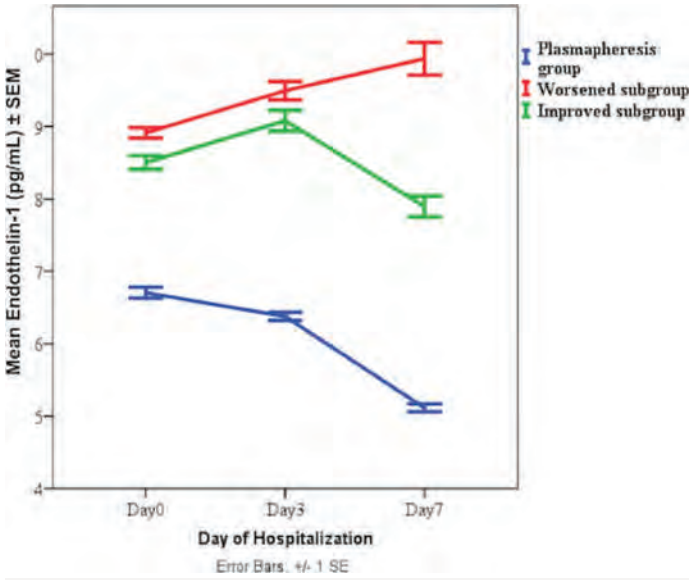

Figure 3. Endothelin-1 trajectories in patients with acute pancreatitis. Mean±SEM levels of endothelin-1 on days 1, 3, and 7 in the plasmapheresis group (n=68), Worsened subgroup (n=17), and Improved subgroup (n=15).

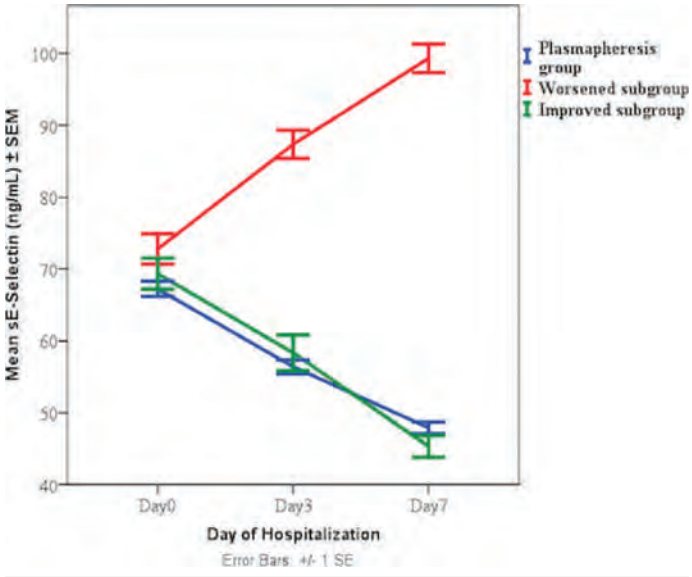

Figure 4. sE-selectin trajectories in patients with acute pancreatitis. Mean±SEM levels of soluble E-selectin (sE-selectin) on days 1, 3, and 7 in the plasmapheresis group (n=68), Worsened subgroup (n=17), and Improved subgroup (n=15).

adjustment for potential confounders, multivariable logistic regression analysis was performed. After adjustment for age, BMI, and admission levels of VEGF, vWF, ET-1, and sE-selectin, plasmapheresis remained independently

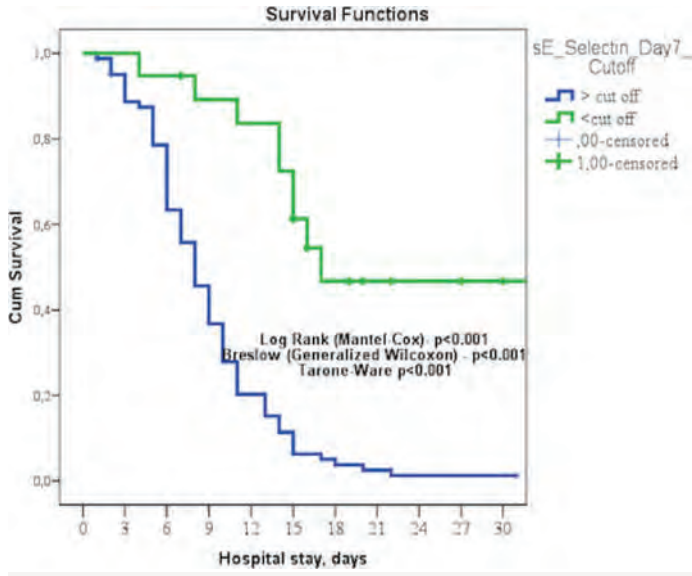

Figure 5. Kaplan–Meier survival curves for patients stratified according to the day 7 sE-selectin cutoff value of 59.5 ng/mL.

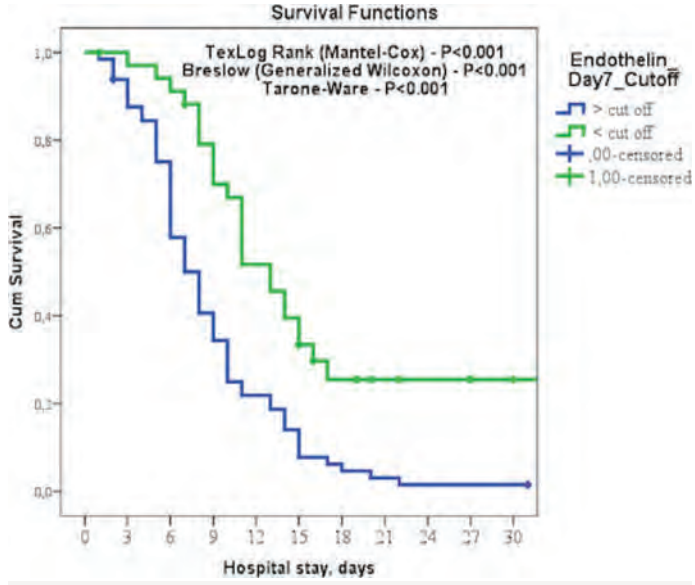

Figure 6. Kaplan–Meier survival curves for patients stratified according to the day 7 endothelin-1 cutoff value of 5.8 pg/mL.

associated with a lower rate of clinical deterioration (adjusted OR=0.21, 95% CI: 0.07–0.58, p=0.002). Among the biomarkers, admission sE-selectin (adjusted OR=1.82 per 10-ng/mL increase, 95% CI: 1.25–2.64, p=0.002) and ET-1 (adjusted OR=1.58 per 1-pg/mL increase, 95% CI: 1.12–2.22, p=0.009) were significant independent predictors of clinical deterioration.

Table 2. Predictive accuracy of endothelial dysfunction biomarkers measured on day 3 for clinical deterioration in acute pancreatitis

Biomarker	AUC	95% CI	Interpretation
VEGF	0.744 (p=0.005)	—	Good predictor
vWF	0.623 (p=0.153)	—	Weak predictor
Endothelin-1	0.771 (p=0.002)	—	Good predictor
sE-selectin	**0.905 (p<0.001)**	—	Excellent predictor

AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; ICU: Intensive care unit; sE-selectin, soluble E-selectin; VEGF: Vascular endothelial growth factor; vWF: von Willebrand factor. Clinical deterioration was defined as a composite endpoint of in-hospital mortality or new or worsening organ failure requiring ICU admission.

Table 3. Predictive accuracy of endothelial dysfunction biomarkers measured on day 7 for clinical deterioration in acute pancreatitis

Biomarker	AUC	95% CI	Interpretation
VEGF	0.790 (p=0.001)	—	Good predictor
vWF	0.746 (p=0.004)	—	Moderate predictor
Endothelin-1	0.847 (p<0.001)	—	Very good predictor
sE-selectin	0.858 (p<0.001)	—	Excellent predictor

AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; ICU: Intensive care unit; sE-selectin, soluble E-selectin; VEGF: Vascular endothelial growth factor; vWF: von Willebrand factor. Clinical deterioration was defined as a composite endpoint of in-hospital mortality or new or worsening organ failure requiring ICU admission.

Predictive Accuracy of Biomarkers

Receiver operating characteristic curve analysis demonstrated that endothelial biomarkers, particularly sE-selectin and ET-1, were strong predictors of clinical deterioration on both day 3 and day 7 (Tables 2 and 3). On day 3, sE-selectin demonstrated excellent predictive accuracy (AUC=0.905, p<0.001), followed by ET-1 (AUC=0.771, p=0.002) and VEGF (AUC=0.744, p=0.005). vWF was a weak predictor on day 3 (AUC=0.623, p=0.153), but its predictive accuracy improved by day 7 (AUC=0.746, p=0.004). The predictive accuracy of most biomarkers improved or remained stable over time. The optimal prognostic cutoff values derived using Youden's index are presented in Table 4.

Table 4. Optimal prognostic cutoff values for endothelial dysfunction biomarkers on days 3 and 7 of hospitalization

Biomarker	Cut-off value on day 3	Cut-off value on day 7
VEGF	102.5 pg/mL	113.7 pg/mL
vWF	85.7 IU/dL	105.3 IU/dL
Endothelin-1	6.9 pg/mL	5.8 pg/mL
sE-selectin	56.9 ng/mL	59.5 ng/mL

ROC: Receiver operating characteristic; sE-selectin, soluble E-selectin; VEGF: Vascular endothelial growth factor; vWF: von Willebrand factor. Optimal cutoff values were determined using Youden's index (J) from ROC analysis for the composite endpoint of clinical deterioration, defined as mortality or multiorgan dysfunction syndrome.

Survival Analysis

Kaplan–Meier analysis using all-cause mortality as the endpoint revealed clear stratification of outcomes according to ET-1 levels (Fig. 6). Patients with day 7 ET-1 levels <5.8 pg/mL had a survival rate of 96.9% and a median survival time of 30.5 days, compared with a survival rate of 68.6% and a median survival time of 22.7 days among those with ET-1 levels ≥5.8 pg/mL (log-rank p<0.001). Similar stratification was observed for sE-selectin (Fig. 5), with patients below the cutoff value of 59.5 ng/mL demonstrating significantly better survival than those above the cutoff (log-rank p<0.001). Cox proportional hazards regression was not performed because the primary aim of this analysis was to visually demonstrate risk stratification using the derived biomarker cutoff values.

DISCUSSION

This study demonstrates that serial endothelial dysfunction biomarkers are strong predictors of clinical deterioration in AP and that their trajectories differed among patients who received adjunctive plasmapheresis. TPE has been shown to improve hemodynamics and survival in sepsis.[12,13] These benefits have also been supported by systematic reviews.[14] Its use is increasingly supported in inflammatory crises, including pediatric sepsis and cytokine storm syndromes, highlighting its potential as an endothelial-stabilizing intervention.[15–17]

We acknowledge that established severity indices, such as the BISAP, APACHE II, CTSI, and SOFA scores, were not calculated in this study. Our primary objective was to evaluate whether a panel of endothelial biomarkers measurable from a single blood sample could provide a more streamlined and accessible approach to early risk stratification in AP while avoiding the complexity and resource requirements of multiparameter scoring systems. Although these established scores have proven value, they are often cumbersome to calculate in real time and require multiple laboratory and clinical parameters that may not be universally available. The absence of these scores limits our ability to fully characterize baseline severity and confirm comparability between the groups. However, baseline biomarker levels were comparable between the plasmapheresis and conventional therapy groups, and our multivariable analysis was adjusted for key baseline indicators, including age, BMI, and admission biomarker levels. Nevertheless, future studies incorporating established severity indices are needed to validate our findings and compare the prognostic performance of endothelial biomarkers with that of these standardized scoring systems.

We evaluated the association of plasmapheresis with clinical outcomes and endothelial dysfunction in patients with AP and compared the findings with those of patients receiving conventional guideline-based therapy. Previous research on plasmapheresis in AP has focused predominantly on hypertriglyceridemia-induced cases, with heterogeneous findings; some studies have reported clinical benefits, whereas others have demonstrated limited efficacy or procedure-related risks.[18–21] Our study differs in that most enrolled patients had biliary or idiopathic pancreatitis, and plasmapheresis was used primarily as a strategy to mitigate endothelial injury rather than rapidly reduce triglyceride levels.

Patients receiving plasmapheresis had more favorable outcomes. Among the 68 patients who received adjunctive therapy, the mortality rate was 1.47%, compared with 6.25% in the conventional therapy group. However, given the nonrandomized design, these differences should be interpreted as associations rather than causal effects. Notably, both deaths in the conventional therapy group occurred in the Worsened subgroup, which comprised patients whose clinical conditions deteriorated despite standard therapy, resulting in a subgroup-specific mortality rate of 11.76%. These findings suggest that early modulation of endothelial dysfunction may attenuate disease progression and reduce the likelihood of multiorgan involvement. Comparable improvements have been described in recent studies, in which plasmapheresis was associated with reductions in circulating inflammatory mediators, endothelial activation markers, and prothrombotic factors, thereby stabilizing microvascular function and accelerating clinical recovery.[9,12,13,16]

Our analysis revealed that the endothelial biomarkers VEGF, vWF, ET-1, and E-selectin were strongly associated with disease severity, organ dysfunction, length of hospitalization, and survival. vWF demonstrated comparatively weaker predictive performance, particularly on day 3 (AUC=0.623, p=0.153), although its accuracy improved by day 7 (AUC=0.746, p=0.004). This finding may be explained by the fact that vWF is an acute-phase reactant released not only from activated endothelial cells but also from platelets and megakaryocytes in response to systemic inflammation, stress, and tissue injury.[4,5] Consequently, vWF levels may be elevated in a broad range of conditions other than endothelial dysfunction, including acute inflammatory states and thrombotic events, which may reduce its specificity as a prognostic marker in AP. In contrast, sE-selectin and ET-1 are more specifically associated with endothelial activation and vascular permeability, which may account for their superior predictive accuracy.

Patients in the conventional therapy group, particularly those in the Worsened subgroup, demonstrated persistently elevated biomarker levels, whereas those who underwent plasmapheresis experienced significant reductions by days 3 and 7. These findings are consistent with previous studies highlighting the central roles of endothelial barrier disruption, microcirculatory thrombosis, and dysregulated angiogenesis in the pathophysiology of severe AP.

The dynamic behavior of biomarkers during treatment provides additional insight. Plasmapheresis was associated with early reductions in VEGF, ET-1, and E-selectin, which are molecules implicated in increased vascular permeability, vasoconstriction, and leukocyte adhesion. Meanwhile, vWF, a marker of endothelial damage and platelet activation, declined more slowly but still showed a downward trend with plasmapheresis. In contrast, patients with clinical deterioration demonstrated increasing levels of these markers, consistent with progressive endothelial injury and microvascular failure. Taken together, our results support the view that endothelial dysfunction is not merely a consequence of AP but may also be a driver of clinical deterioration.

Overall, the findings indicate that plasmapheresis may mitigate the endothelial component of AP pathophysiology, thereby reducing complications and improving outcomes. This interpretation is consistent with growing evidence suggesting that targeting endothelial stabilization through extracorporeal blood purification or pharmacologic interventions may represent a promising adjunctive therapeutic strategy for severe forms of the disease.

Strengths and Limitations

Strengths

The prospective study design, with serial measurements obtained on days 1, 3, and 7, allowed a detailed evaluation of biomarker dynamics.

The comprehensive endothelial biomarker panel, including VEGF, vWF, ET-1, and E-selectin, provided a mechanistic perspective beyond that offered by classical inflammatory markers.

The inclusion of clinical outcomes, such as organ failure, ICU stay, and mortality, enabled a meaningful assessment of prognostic performance.

The use of multimodal statistical approaches, including ROC curve analysis and repeated-measures ANOVA, ensured a robust interpretation of biomarker significance.

The novel application of plasmapheresis in biliary and non-hypertriglyceridemic pancreatitis adds original value to the existing literature.

Limitations

Several limitations of this study should be acknowledged. First, allocation to plasmapheresis was nonrandomized and based on clinical discretion, introducing a substantial risk of selection bias and confounding by indication. Although multivariable adjustment was performed for baseline severity indicators, unmeasured confounding cannot be entirely excluded. Therefore, all observed associations between plasmapheresis and clinical outcomes should be interpreted as associative rather than causal.

Second, the single-center design limits the generalizability of our findings to broader populations and different healthcare settings. External validation in multicenter cohorts is necessary before the proposed biomarker cutoff values can be adopted in clinical practice.

Third, although the sample size was adequate for the primary analyses, it may have been insufficient to detect subtle subgroup differences or permit robust derivation of cutoff values. The post hoc subdivision of the conventional therapy cohort further limits comparability among the groups.

Fourth, the absence of long-term follow-up limited the evaluation of delayed complications and recurrent pancreatitis.

Fifth, biomarker assays were performed using kits from a single manufacturer, which may limit comparability across studies, although internal consistency was strong.

Accordingly, all observed associations between plasmapheresis and clinical outcomes should be interpreted as associative rather than causal, and no therapeutic claims can be made based on these data. Randomized controlled trials are needed to determine whether plasmapheresis has a causal protective effect.

Furthermore, established severity indices, such as the BISAP, APACHE II, CTSI, and SOFA scores, were not calculated, limiting our ability to fully characterize baseline disease severity and confirm group comparability beyond the measured biomarkers. This limitation should be addressed in future validation studies.

Additionally, Cox proportional hazards regression was not performed to estimate adjusted hazard ratios for mortality. Although our Kaplan–Meier analysis visually demonstrated the prognostic value of biomarker cutoff values, future studies should incorporate Cox regression to provide adjusted effect estimates and account for potential confounders.

Finally, the exploratory nature of the analyses, involving multiple comparisons across several biomarkers and time points, increased the risk of type I error. Accordingly, our findings should be considered hypothesis-generating and require confirmation in prospective, randomized, and adequately powered trials.

CONCLUSION

Serial endothelial dysfunction biomarkers, particularly sE-selectin and ET-1, are strong early predictors of clinical deterioration in acute pancreatitis. Plasmapheresis was associated with favorable biomarker trajectories and significantly lower rates of clinical deterioration than those observed in the Worsened subgroup of patients receiving conventional therapy alone. These findings support the role of endothelial biomarkers in early risk stratification and suggest that endothelial dysfunction may represent a modifiable therapeutic target. Randomized controlled trials are needed to confirm these associations and establish the efficacy of biomarker-guided plasmapheresis in acute pancreatitis.

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Comparison of the Diagnostic Efficacy of Frameless Stereotactic Biopsy (Varioguide) with Other Methods Reported in the Literature: A Single-Center Experience

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ABSTRACT

Objective: To evaluate the diagnostic yield, complication profile, and demographic and radiological factors associated with procedural success in intracranial biopsies performed using the VarioGuide frameless stereotactic system.

Materials and Methods: We retrospectively reviewed 58 consecutive patients who underwent VarioGuide-assisted frameless stereotactic brain biopsy between August 2018 and December 2025. All procedures were performed under general anesthesia using co-registered preoperative high-resolution computed tomography and magnetic resonance imaging datasets. The associations of age, sex, lesion volume, and contrast-enhancement pattern with diagnostic success and postoperative complications were evaluated using exploratory univariable analyses.

Results: The cohort included 42 male (72.4%) and 16 female (27.6%) patients, with a mean age of 49.8±18.7 years. The diagnostic yield was 94.8% (55/58). The median age was 51 years (range, 6–79 years) among patients with diagnostic biopsies and 28 years (range, 2–41 years) among those with non-diagnostic biopsies (exact Mann–Whitney U test, p=0.020). Lesion volume (p=0.843), sex (p=0.181), and contrast-enhancement pattern (p>0.999) were not significantly associated with diagnostic success. Postoperative findings occurred in 10 patients (17.2%), including asymptomatic microhemorrhage in six (10.3%), pneumocephalus in two (3.4%), and symptomatic hematomas requiring surgical decompression in two (3.4%). Neither age (p=0.133) nor lesion volume (p=0.951) differed significantly according to complication status.

Conclusion: In this retrospective single-center series, VarioGuide-assisted frameless stereotactic biopsy provided a high diagnostic yield and a low rate of major procedure-related morbidity. Younger age was associated with a non-diagnostic biopsy in an exploratory analysis; however, this finding should be interpreted cautiously because only three biopsies were non-diagnostic.

Keywords: Associated factors, brain biopsy, complications, diagnostic yield, frameless stereotaxy, varioguide.

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INTRODUCTION

In the management of intracranial lesions, establishing a definitive histopathological diagnosis is the gold standard for determining the optimal treatment strategy.[1,2] Stereotactic biopsy plays a critical role, particularly in the diagnosis of lesions that are deeply located, adjacent to eloquent areas, multiple, or unsuitable for surgical resection.[3] Conventional frame-based stereotactic systems, which have been used for many years with high accuracy, have disadvantages such as patient discomfort caused by the rigid frame, logistical challenges between preoperative imaging and surgery, and prolonged operative times.[4,5] Frameless neuronavigation systems developed to overcome these limitations have gained an important role in clinical practice.[6,7] Systems such as VarioGuide (Brainlab, Munich, Germany) may streamline the operative workflow and allow more flexible use of preoperative imaging.[8] Frameless systems have been reported to provide diagnostic yields and accuracy rates comparable to those of frame-based systems.[9,10] This retrospective study aimed to evaluate the diagnostic yield, postoperative morbidity, and demographic and radiological factors associated with diagnostic success and complications in a single-center series of VarioGuide-assisted frameless stereotactic biopsies.

KEY MESSAGES

- In this retrospective single-center series of 58 patients, VarioGuide-assisted frameless stereotactic biopsy achieved a diagnostic yield of 94.8% (55/58).
- Younger age was associated with a non-diagnostic biopsy in an exploratory comparison, whereas lesion volume, sex, and contrast-enhancement pattern were not significantly associated with diagnostic success; however, this finding should be interpreted cautiously because only three biopsies were non-diagnostic.
- Procedure-related complications occurred in 17.2% of patients and were predominantly minor and asymptomatic; major hematomas requiring surgical decompression occurred in 3.4%, and no procedure-related mortality was observed.

MATERIALS AND METHODS

Study Site, Design, and Study Period

This retrospective, single-center clinical study was conducted at Erciyes University. We retrospectively reviewed all consecutive patients who underwent frameless stereotactic brain biopsy using the VarioGuide system (Brainlab, Munich, Germany) between August 2018 and December 2025. The study was designed to evaluate diagnostic yield, surgical morbidity, complication profiles, and demographic and radiological factors associated with procedural success.

Ethics Approval

The study was approved by Erciyes University Clinical Research Ethics Committee (Approval Number: 2026/211, Date: 22.04.2026) and was conducted in accordance with the Declaration of Helsinki.

Sample Size

No a priori sample size calculation was performed because this was a retrospective consecutive case series. All patients who met the eligibility criteria during the predefined study period were included. Because the numbers of non-diagnostic biopsies and postoperative complications were small, the comparative analyses were considered exploratory.

Patients and Data Collection

Demographic information, preoperative radiological examinations, surgical notes, early postoperative CT images, and definitive histopathology reports were obtained from the hospital automation system. Patient age, sex, lesion volume, lesion location, contrast-enhancement pattern, histopathological diagnosis, diagnostic success, and postoperative complications were recorded for analysis.

Diagnostic Criteria

The final diagnosis was based on the histopathological examination of the biopsy specimens. Diagnostic success was defined as the acquisition of sufficient tissue to establish a definitive histopathological diagnosis. Diagnostic yield was calculated as the proportion of patients in whom a definitive histopathological diagnosis was obtained among all patients who underwent biopsy.

Definitions

Lesion location was categorized according to the main anatomical region involved. Contrast enhancement on preoperative MRI was classified as homogeneous, heterogeneous, or absent. Lesion volume was assessed preoperatively using the navigation and planning software and recorded in cubic centimeters (cm^3). Postoperative complications were evaluated using early postoperative CT findings and clinical records. Major morbidity was defined as a symptomatic hematoma requiring surgical decompression. A non-diagnostic biopsy was defined as failure to establish a definitive histopathological diagnosis after the biopsy procedure.

Inclusion Criteria

Patients were included if they required tissue diagnosis of an intracranial mass, were considered unsuitable for surgical

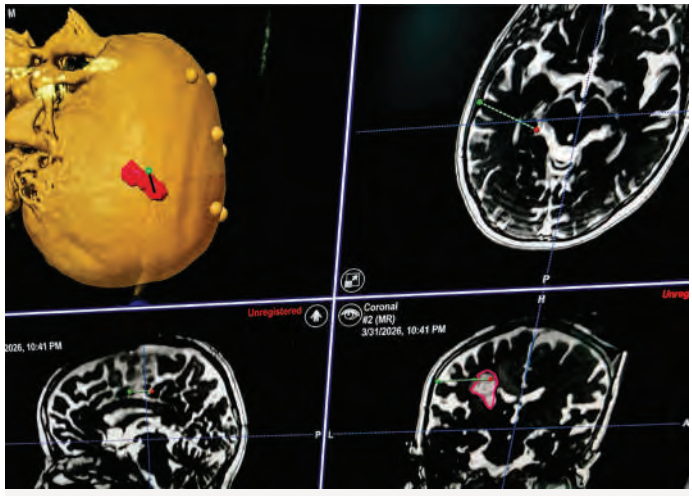

Figure 1. Preoperative planning of a thalamic lesion using neuronavigation software. The target and entry points determined after CT/MRI fusion (co-registration) are shown in the axial, coronal, and sagittal planes. In the three-dimensional reconstruction model in the upper-left panel, the projection of the lesion (red) onto the cranial anatomy and the safest planned surgical trajectory (green line) toward the target are shown.

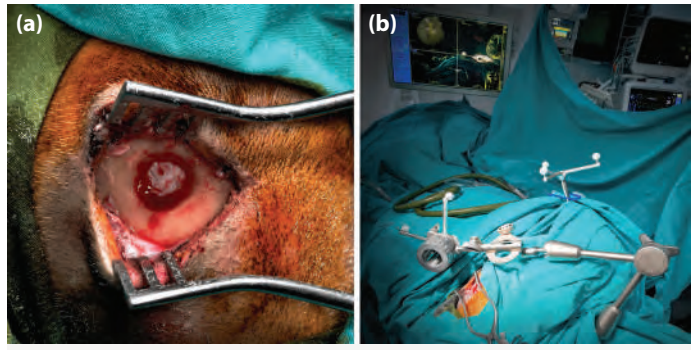

Figure 2. Intraoperative surgical setup and view of the VarioGuide system. **(a)** Macroscopic view of the single burr hole and dural opening created at the planned entry point. **(b)** Operating room setup and integration of the navigation system. The patient's head is secured in a Mayfield skull clamp, and the optical reference stars are registered with the system. The VarioGuide arm (lower center) is integrated into the navigation system and locked along the predetermined surgical trajectory. The optical camera and navigation screen, which provide real-time tracking during the procedure, are visible in the background.

resection because the lesion was deeply located, adjacent to eloquent areas, or multiple, and underwent biopsy using the VarioGuide frameless stereotactic system. Patients with available preoperative MRI/CT data, postoperative CT imaging, surgical records, and histopathological follow-up were included in the analysis.

Exclusion Criteria

Patients whose complete datasets, including preoperative MRI/CT and postoperative CT images, could not be accessed were excluded. Patients with severe coagulopathy or whose antiplatelet or anticoagulant therapy could not be discontinued within a safe period before surgery were also excluded. In addition, cases in which the acceptable registration error margin of 2 mm was exceeded during surgery and cases with incomplete histopathological follow-up were excluded from the evaluation.

Clinical, Surgical, and Laboratory Examinations

All patients underwent preoperative clinical and radiological evaluations. Preoperative magnetic resonance imaging (MRI), including thin-slice contrast-enhanced T1-weighted and T2/FLAIR sequences, was performed. For surgical planning, volumetric computed tomography (CT) suitable for the navigation protocol was obtained. The CT images were transferred to the navigation planning software (Brainlab Elements/iPlan, Brainlab AG) and fused (co-registered) with the preoperative MR images. Using the fused images, the lesion target and entry points were determined, and the safest surgical trajectory that avoided vascular structures, sulci, and ventricles was planned (Fig. 1). Patients underwent routine preoperative laboratory testing, and those with severe coagulopathy were excluded from the study.

All procedures were routinely performed under general anesthesia. The patient's head was secured in a Mayfield pin head-holder system with an integrated navigation reference star. The optical camera system was positioned, and patient registration was completed using anatomical landmarks and surface matching. After registration accuracy was confirmed, the VarioGuide arm was integrated into the navigation system, and instrument calibration was performed (Fig. 2). A linear skin incision was made, followed by the creation of a single burr hole at the planned entry point. After the dura mater was coagulated and incised, the VarioGuide arm was locked along the preplanned trajectory. A Sedan-type biopsy needle was advanced under navigation guidance to the predetermined target depth, and serial samples were obtained from different quadrants through the needle tip (Fig. 3). The samples were sent to the pathology laboratory for histopathological examination. Routine postoperative brain CT was performed in all patients to rule out complications such as hemorrhage or pneumocephalus.

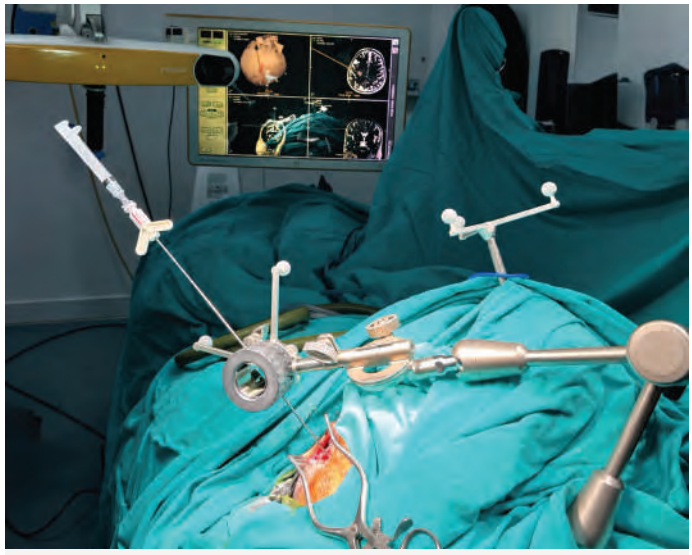

Figure 3. Intraoperative biopsy and tissue sampling. The calibrated Sedan-type stereotactic biopsy needle is advanced to the preplanned target depth in the thalamus under VarioGuide guidance. The image shows the needle being directed toward the intracranial target along the fixed trajectory established by the navigation system. Tissue sampling is performed while monitoring the real-time position of the needle tip and its distance from the target on the navigation screen in the background.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were summarized as the mean±standard deviation or median (minimum–maximum), as appropriate, and categorical variables were summarized as frequencies and percentages. Age and lesion volume were compared between two independent groups using the two-sided Mann–Whitney U test; exact significance values were used in analyses of diagnostic success because the non-diagnostic group included only three patients. Categorical variables were compared using Fisher's exact test for 2×2 tables and the Fisher–Freeman–Halton exact test for larger contingency tables. Anatomical location was summarized descriptively and was not analyzed inferentially because only three non-diagnostic events were distributed across eight location categories. No adjustment was made for multiple comparisons. A two-sided p value of <0.05 was considered statistically significant.

RESULTS

Demographic and Clinical Characteristics

Of the 58 patients included in the study, 42 were male (72.4%) and 16 were female (27.6%). The mean age of the cohort was 49.81±18.67 years (range, 2–79 years). Radiological evaluation showed a mean lesion volume of 15.12±15.39 cm^3 (range, 1–62 cm^3). Heterogeneous contrast enhancement was observed in 27 lesions (46.6%), homogeneous enhancement in seven (12.1%), and no contrast enhancement in 24 (41.4%) (Table 1).

Table 1. Demographic, clinical, and radiological characteristics of the study population

Variable	n (%) / Mean±SD
Sex	
Male	42 (72.4)
Female	16 (27.6)
Age (years)	
Mean±SD	49.81±18.67
Minimum–maximum	2–79
Lesion location	
Frontal	15 (25.9)
Thalamic	13 (22.4)
Temporal	13 (22.4)
Parietal	12 (20.7)
Occipital	2 (3.4)
Pineal	1 (1.7)
Mesencephalic	1 (1.7)
Intraventricular	1 (1.7)
Contrast enhancement	
Heterogeneous	27 (46.6)
None	24 (41.4)
Homogeneous	7 (12.1)
Lesion volume (cm^3)	
Mean±SD	15.12±15.39

Data are presented as number (percentage) for categorical variables and mean±standard deviation for continuous variables. cm^3, cubic centimeters; n, number of patients; SD: Standard deviation.

The most common lesion location was the frontal lobe (n=15, 25.9%), followed by the thalamus (n=13, 22.4%), temporal lobe (n=13, 22.4%), and parietal lobe (n=12, 20.7%). The remaining lesions were located in the occipital lobe (n=2, 3.4%), mesencephalon (n=1, 1.7%), pineal region (n=1, 1.7%), and lateral ventricle (n=1, 1.7%) (Table 1).

Histopathological Diagnoses

Histopathological analysis revealed diffuse astrocytoma as the most common diagnosis (n=15, 25.9%), followed by high-grade glial tumors (n=10, 17.2%), low-grade glial tumors (n=6, 10.3%), and glioblastoma (n=6, 10.3%). B-cell lymphoma (n=5, 8.6%) and oligodendroglioma (n=5, 8.6%) were also identified.

Table 2. Factors associated with diagnostic success

Variable	Diagnostic biopsy (n=55)	Non-diagnostic biopsy (n=3)	Statistical test	p
Age, years, median (minimum–maximum)	51 (6–79)	28 (2–41)	Exact Mann–Whitney U test	0.020
Lesion volume, cm^3, median (minimum–maximum)	9 (1–62)	7 (6–28)	Exact Mann–Whitney U test	0.843
Sex, n (%)			Fisher's exact test	0.181
Male	41 (74.5)	1 (33.3)		
Female	14 (25.5)	2 (66.7)		
Contrast enhancement, n (%)			Fisher–Freeman–Halton exact test	>0.999
None	23 (41.8)	1 (33.3)		
Heterogeneous	25 (45.5)	2 (66.7)		
Homogeneous	7 (12.7)	0 (0.0)		

Data are presented as median (minimum–maximum) or n (%). Exact two-sided p values were used where indicated. Anatomical location was summarized descriptively and was not analyzed inferentially because only three non-diagnostic events were distributed across eight location categories. cm^3, cubic centimeters; n, number of patients; p, probability value.

Table 3. Factors associated with postoperative complications

Variable	Complication (n=10)	No complication (n=48)	Statistical test	p
Age, years, median (minimum–maximum)	65 (28–79)	48 (2–76)	Mann–Whitney U test	0.133
Lesion volume, cm^3, median (minimum–maximum)	14.5 (2–28)	8.5 (1–62)	Mann–Whitney U test	0.951

Data are presented as median (minimum–maximum). p values were calculated using two-sided Mann–Whitney U tests. cm^3, cubic centimeters; n, number of patients; p, probability value.

Diagnostic Yield and Associated Factors

The overall diagnostic yield was 94.8% (55/58), with three biopsies being non-diagnostic. Two of the non-diagnostic lesions were located in the frontal lobe, and one was located in the temporal lobe. Anatomical location was summarized descriptively and was not subjected to inferential testing because only three non-diagnostic events were distributed across eight location categories.

Patients with diagnostic biopsies had a median age of 51 years (range, 6–79 years), compared with 28 years (range, 2–41 years) among patients with non-diagnostic biopsies (exact Mann–Whitney U test, p=0.020). The median lesion volume was 9 cm^3 (range, 1–62 cm^3) in the diagnostic group and 7 cm^3 (range, 6–28 cm^3) in the non-diagnostic group (p=0.843). Diagnostic success was not significantly associated with sex (Fisher's exact test, p=0.181) or contrast-enhancement pattern (Fisher–Freeman–Halton exact test, p>0.999). Because there were only three non-diagnostic cases, these comparisons were interpreted as exploratory (Table 2).

Surgical Complications and Associated Factors

Procedure-related postoperative findings were recorded in 10 patients (17.2%), including asymptomatic microhemorrhage in six (10.3%), pneumocephalus in two (3.4%), and symptomatic hematoma requiring surgical decompression in two (3.4%). No procedure-related mortality occurred.

The median age was 65 years (range, 28–79 years) among patients with complications and 48 years (range, 2–76 years) among those without complications (Mann–Whitney U test, p=0.133). The median lesion volumes were 14.5 cm^3 (range, 2–28 cm^3) and 8.5 cm^3 (range, 1–62 cm^3), respectively (p=0.951) (Table 3).

DISCUSSION

Histopathological diagnosis remains central to selecting appropriate oncological and surgical treatments for intracranial lesions. In this retrospective series of 58 VarioGuide-assisted frameless stereotactic biopsies, the overall diagnostic yield was 94.8%. This result is within the 91%–99% range reported in contemporary frameless and frame-based stereotactic biopsy series and supports the clinical utility of frameless navigation-guided biopsy systems.[9–12]

In exploratory analyses, younger age was associated with a non-diagnostic biopsy. However, only three non-diagnostic procedures occurred, and this finding should not be interpreted as an established risk factor. Lesion volume, sex,

and contrast-enhancement pattern were not significantly associated with diagnostic success. The three non-diagnostic lesions were located in the frontal (n=2) and temporal (n=1) regions; therefore, lesion location was reported descriptively rather than analyzed inferentially. Larger multicenter series are required to determine whether age or lesion location independently influences diagnostic yield.

Postoperative hemorrhage is the most feared complication of stereotactic brain biopsy and directly contributes to surgical morbidity. In the literature, asymptomatic microhemorrhages have been reported at rates of up to 10%, whereas symptomatic hematomas have been reported at rates ranging from 1% to 6%.[13,14]

Although postoperative findings were recorded in 17.2% of patients, most consisted of asymptomatic microhemorrhages (n=6, 10.3%) or pneumocephalus (n=2, 3.4%) without clinical deficits. Symptomatic hematomas requiring surgical decompression occurred in two patients (3.4%), and no procedure-related mortality was observed. These findings are consistent with the reported morbidity profile of stereotactic brain biopsy.[14,15]

The absence of procedure-related mortality and the low number of major complications support an acceptable safety profile in this cohort. Nevertheless, the absence of statistically significant associations should not be interpreted as evidence that age or lesion volume has no effect because only 10 postoperative events were available for comparison.[15]

The literature suggests that necrotic or fragile vascular structures in very large lesions or multiple needle passes required to reach the target in very small lesions may increase the risk of bleeding.[16]

In our cohort, age and lesion volume did not differ significantly between patients with and without postoperative complications (p=0.133 and p=0.951, respectively). These exploratory comparisons suggest that no clear association was detectable in the present sample; however, the limited number of events reduced statistical power and precluded reliable multivariable modeling.

One of the most common criticisms of frameless systems is the risk of missing the target, particularly in very small or non-enhancing lesions.[17,18]

Diagnostic success was not significantly associated with lesion volume (p=0.843) or contrast-enhancement pattern (p>0.999). Twenty-four lesions (41.4%) showed no contrast enhancement, and the overall diagnostic yield remained high. These observations suggest that VarioGuide-assisted biopsy may be clinically useful across heterogeneous radiological presentations; however, they do not establish equivalent performance across lesion subgroups because the non-diagnostic group was very small.

Limitations

This study has several limitations. Its retrospective, single-center design may introduce selection and information biases and limit generalizability. No a priori power calculation was performed because all eligible consecutive patients were included. The small numbers of non-diagnostic biopsies (n=3) and postoperative complications (n=10) limited statistical power, precluded reliable multivariable modeling, and increased the possibility of unstable estimates. Therefore, the observed association between younger age and a non-diagnostic biopsy should be considered exploratory and requires confirmation in larger cohorts. In addition, variables such as lesion consistency, the number of tissue samples, and minor differences in surgical technique could not be quantitatively evaluated.

CONCLUSION

In this retrospective single-center series, VarioGuide-assisted frameless stereotactic biopsy provided a high diagnostic yield and a low rate of major procedure-related morbidity. Younger age was associated with a non-diagnostic biopsy in an exploratory analysis, whereas lesion volume, sex, and contrast-enhancement pattern were not significantly associated with diagnostic success. These findings support the clinical utility of the system but should be interpreted cautiously because of the small number of non-diagnostic cases.

Ethics Committee Approval: Ethics committee approval was obtained from Erciyes University Clinical Research Ethics Committee (Approval Number: 2026/211, Date: 22.04.2026).

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Intravenous Thrombolysis for Stroke in First-Trimester Pregnancy: A Case Report

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ABSTRACT

Background: The use of thrombolytic therapy during pregnancy remains a source of concern and stress. In pregnant patients who meet the criteria, thrombolytic therapy is recommended when the expected benefits outweigh the risks.

Case Report: A 33-year-old pregnant woman in her first trimester presented with an acute-onset impairment of speech and comprehension. Her initial National Institutes of Health Stroke Scale score was 4. Diffusion MRI was compatible with cortical-subcortical acute ischemia in the left frontotemporal region. Intravenous thrombolytic therapy was administered to the patient who met the criteria for thrombolysis. No bleeding or other complications were observed after treatment. On examination, after one week, all symptoms had completely resolved. The pregnancy ended in fetal abortion at 16 weeks; the cause was not determined.

Conclusion: Publication of case reports on the complications and efficacy of IVT during pregnancy is important, since there are no controlled studies of thrombolytic therapy in pregnant women.

Keywords: Cerebrovascular disease, intravenous thrombolysis, pregnancy, recanalization therapy, stroke.

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INTRODUCTION

Acute cerebrovascular disease is a rare condition in pregnancy, however, it can have serious consequences such as maternal death or disability.[1,2] The most common cause of disability following pregnancy is cerebrovascular disease.[3] Prothrombotic state, gestational diabetes, eclampsia, and pre-eclampsia in pregnancy increases the risk of cerebrovascular disease in addition to other known vascular risk factors.[2,3]

Intravenous thrombolysis (IVT) is the current recommended treatment for ischemic stroke in eligible patients.[4] Managing stroke treatment during pregnancy is stressful. Due to the lack of randomized controlled studies involving pregnant patients, there is limited knowledge about the safety and effectiveness of thrombolytic therapy.[4-6] Concern about damage to the fetus or mother may cause physicians to avoid thrombolytic therapy.[7] It is important to publish case reports and complications or efficacy of IVT in pregnancy to ensure additional information and to provide resources for retrospective studies.[6,8]

CASE REPORT

A 33-year-old woman at six weeks' gestation presented to the emergency department with sudden-onset slurred speech, word-finding difficulty, and impaired comprehension that began 80 minutes earlier. Her vital signs were stable, with a blood pressure of 100/70 mmHg. Aside from mild mixed aphasia and slight dysarthria, neurological examination revealed no other findings, such as cranial nerve disorder or paralysis; the National Institutes of Health stroke score was 4.

Her medical history included hypothyroidism, but no known vascular risk factors were present. The standard electrocardiogram was normal. Laboratory parameters did not demonstrate any abnormalities, including a platelet count of 245 $\times 10^3$/µL, a partial thromboplastin time (aPTT) of 32.6 s, a prothrombin time of 11 s, and an International Normalized Ratio (INR) of 0.94.

Computed tomography (CT) of the brain was not preferred for neuroimaging because of pregnancy. It was readily possible to perform an MRI. A diffusion MRI showed diffusion restriction consistent with cortical-subcortical acute ischemia in the left frontotemporal region. Brain magnetic resonance imaging (MRI) findings were normal, with no signs of intracranial hemorrhage (Fig. 1, 2). Angiographic imaging, including CT and MR angiography, was not conducted because of a distal branch occlusion and was not available for mechanical thrombectomy. Furthermore, MR angiography is time-consuming, and CT angiography poses a radiation risk to the fetus.

In light of this information, the absence of absolute contraindications, the treatment process, and the benefits and risks are comprehensively explained to the patient's relatives, since communication with the patient cannot be established due to aphasia. After the relatives of the patient preferred intravenous rt-PA (alteplase) administration, intravenous alteplase was administered in accordance with established treatment guidelines: 0.9 mg/kg alteplase; 10% IV push and the remainder infused over 1 hour. On examination after 24 hours, the NIHSS score had decreased to two points; after seven days, she had made a complete recovery. No cerebral, vaginal, or intrauterine hemorrhages were observed after thrombolytic therapy. An obstetric USG performed after thrombolytic treatment was normal. Acetylsalicylic acid at a dose of 100 mg/day was initiated 24 hours following rt-PA administration.

Further investigations were carried out to determine stroke etiology. Serum markers for thrombophilia and an autoimmune screen were unremarkable. Doppler studies of the carotid and vertebral arteries were normal. Cardiac

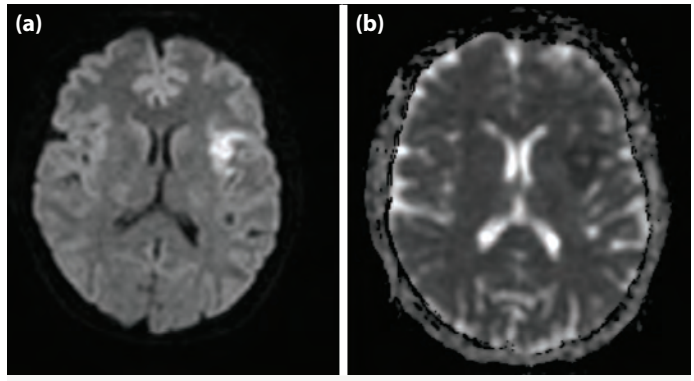

Figure 1. Brain magnetic resonance imaging: **(a)** Diffusion-weighted axial images, **(b)** Apparent diffusion coefficient.

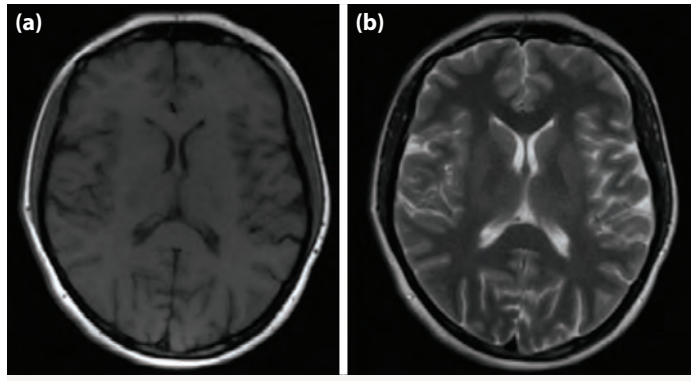

Figure 2. Brain magnetic resonance imaging: **(a)** T1-weighted axial images, **(b)** T2-weighted axial images.

evaluation, including transthoracic echocardiography and extended Holter monitoring, showed no abnormalities. She had no known risk factors for stroke other than pregnancy, and the etiology of the stroke was not determined. Serial obstetric ultrasonography was unremarkable, and the non-invasive prenatal DNA test was negative. The pregnancy ended in a spontaneous miscarriage at the 16th week of gestation, and upon obstetric evaluation, no cause was identified. She had no previous miscarriages and no known risk factors for miscarriage. This was her second pregnancy, and the first resulted in a healthy birth. Obstetric follow-up was normal for 10 weeks after IVT, and it was not thought to be related to IVT.

DISCUSSION

Intravenous thrombolysis is the current suggested treatment of acute ischemic strokes, in those meeting administration criteria.[4] Stroke treatment management is difficult in pregnant women due to concerns about the safety of radiological examinations and the risk of complications of revascularization treatments.[1] Since pregnant women

are excluded from randomized controlled trials of IVT, the effectiveness and potential risks of thrombolytic treatment are poorly understood. Most publications are either case reports or small series, and there have been few retrospective registry-based case-control studies of IVT during pregnancy.[4-6,8]

According to the American Stroke Association recommendation (class IIb), and given limited data, alteplase can be used during pregnancy if the expected benefit in treating moderate or severe stroke outweighs the potential risk of uterine bleeding.[4] The European Stroke Organization (ESO) guidelines on stroke in women do not provide evidence-based recommendations on IVT during pregnancy due to the absence of randomized clinical trials. Experts agree that IV thrombolysis may be administered during pregnancy, provided the patient is eligible and a careful risk-benefit evaluation is conducted in each case.[5]

An important concern in medical treatment during pregnancy is whether it is teratogenic. Rt-PA is unable to cross the placental barrier because of its large molecular weight, and it has not shown teratogenic effects in animals. An embryocidal effect has been observed at high doses in some animal studies, but not at 1 mg/kg, and it is classified as pregnancy category C.[1] Fetal prognosis may result in a healthy birth in most cases, in others abortion have also been reported.[3,6,8]

Brain MRI is the preferred examination for patients with suspected cerebrovascular disease during pregnancy and is not associated with an increased fetal risk. If a brain MRI cannot be performed promptly, a CT scan should be performed. Since the radiation rate of CT on the fetus is at an acceptable level during maternal head and neck examination, it can be used with abdominal protection in pregnant patients with stroke.[2,7,9]

In retrospective studies, there were no differences in intracranial or systemic hemorrhages between pregnant and recently postpartum patients treated with IVT and non-pregnant women. Although there was reported in some studies an increase in symptomatic intracranial hemorrhage in pregnant and postpartum patients, no major systemic bleeding or in-hospital deaths were observed.[10] The short-term outcome and the risk of complications appear to be similar in pregnant and non-pregnant IS patients treated with IVT.[6,8,10]

CONCLUSION

IVT may be offered to pregnant patients when its potential benefits outweigh the anticipated risks or adverse effects. If MRI is not available, CT can be used for neuroimaging in pregnant patients with suspected cerebrovascular disease. Since controlled studies cannot be conducted in pregnant women for ethical reasons, case reports are important for determining the benefits and risks of thrombolytic therapy.

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A Rare Case in a Child: *Actinomycosis* of the Middle Ear

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ABSTRACT

Background: *Actinomyces* is an anaerobic, filamentous bacterium that causes granuloma formation and suppurative infection. Although it is part of the normal flora of the oropharynx, gastrointestinal tract, and genital tract, it is rarely encountered in the middle ear and mastoid cavity.

Case Report: We present the case of a 4-year-old child diagnosed with chronic otitis media who had experienced ear pain and discharge for more than 3 months and whose symptoms did not resolve despite antibiotic therapy. *Actinomyces* was identified histopathologically in a specimen obtained from the middle ear during endoscopic surgery.

Conclusion: This report aims to emphasize that actinomycosis should be considered in the differential diagnosis of patients with chronic suppurative otitis media who do not improve with medical treatment.

Keywords: *Actinomyces*, child, chronic otitis, middle ear, treatment.

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INTRODUCTION

Actinomyces is a gram-positive, anaerobic, non-acid-fast, filamentous bacterium.[1] The most commonly affected sites include the head and neck, abdomen, thorax, and pelvis. Actinomycosis may also occur in the middle ear, with more than half of the reported cases affecting patients younger than 15 years.[2,3] Middle ear contamination may occur after mucosal damage caused by trauma, such as tooth extraction or dental caries; the infection can spread from the nasopharynx to the middle ear via the Eustachian tube. Furthermore, *Actinomyces* has been detected with increasing frequency in the nasopharynx of children with recurrent otitis media and can cause infection through mucosal damage. Another route is direct spread from the external auditory canal to the middle ear through damage to the tympanic membrane. In very rare cases, actinomycosis may also occur via hematogenous spread.[3,4]

Actinomyces forms a fibrous, pseudocapsulated inflammatory granuloma characterized by yellow sulfur granules. A careful medical history, a history of trauma, clinical and radiological findings,

and the identification of *Actinomyces* sulfur granules in patient specimens are crucial for diagnosis.[5]

In this report, we present the case of a 4-year-old child with a long history of ear pain and discharge, in whom histopathological examination of a specimen obtained from the middle ear during endoscopic surgery revealed *Actinomyces*.

CASE REPORT

A 4-year-old girl with no known underlying disease developed pain and discharge in the right ear 3.5 months before presentation. She was treated with antibiotics for a presumptive diagnosis of otitis; however, her symptoms did not completely resolve. Her ear pain and discharge improved during antibiotic treatment but recurred after treatment was discontinued. Temporal bone and inner ear computed tomography (CT) was reported as follows: "Loss of aeration in the right mastoid air cells; soft tissue densities compatible with otomastoiditis in the right mastoid air cells and middle ear. The right middle ear ossicles and inner ear structures appear normal. The left ear structures are normal." An endoscopic ear examination performed by an otorhinolaryngologist, with a preliminary diagnosis of cholesteatoma, revealed a lesion perforating the tympanic membrane and extending into the deeper middle ear spaces that was not externally visible. The patient underwent mastoidectomy with excision of the polypoid lesion, and the tympanic membrane was repaired during the procedure. Pathological examination reported "Inflammatory exudate in the middle ear, chronic inflammation, inflammatory granulation tissue, and gram-positive filamentous bacilli arranged in a fine, radial, and branching pattern with sulfur granules in the center, consistent with *Actinomyces* colonies" (Fig. 1, 2).

During our initial physical examination, the patient was in good general condition, and the systemic examination was unremarkable. No pathology was detected on oropharyngeal or ear examination. Laboratory evaluation revealed a white blood cell count of 7320/µL, an absolute neutrophil count of 2850/µL, an absolute lymphocyte count of 3620/µL, a hemoglobin level of 11.8 g/dL, and a platelet count of 274,000/µL. Liver and kidney function tests were normal. The erythrocyte sedimentation rate was 17 mm/hour (range, 0–20), and the C-reactive protein level was 1.61 mg/L (range, 0–8). Chest radiography was normal.

Middle ear actinomycosis was considered, and high-dose intravenous penicillin G therapy (300,000 U/kg/day in 4 doses) was initiated. Abdominal ultrasonography and brain magnetic resonance imaging were performed to assess organ involvement and revealed no abnormalities. Baseline immunological investigations were normal. The treatment regimen consisted of intravenous penicillin G for 2 weeks, followed by oral penicillin V (100,000 U/kg/day in 4 doses) for an additional 3 months. Post-treatment otoscopic examination was normal. The patient's symptoms resolved. The patient has been followed for 9 months after treatment and has had no complaints. Written informed consent was obtained from the patient's legal guardians for the publication of this case report and the accompanying images.

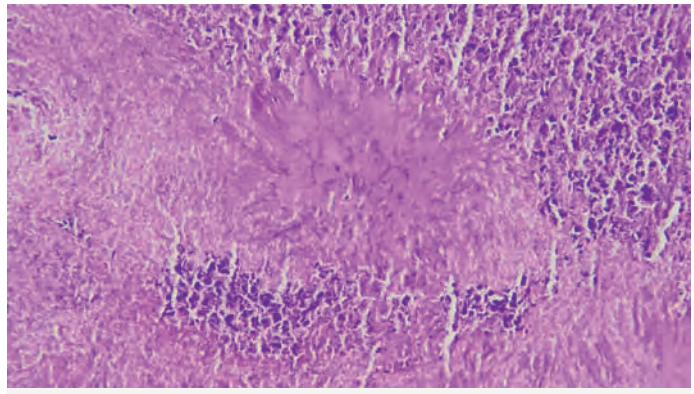

Figure 1. Central sulfur granules formed by a fine, radial, and branching arrangement of gram-positive filamentous bacilli, with *Actinomyces* colonies and inflammatory cells at the periphery (×40).

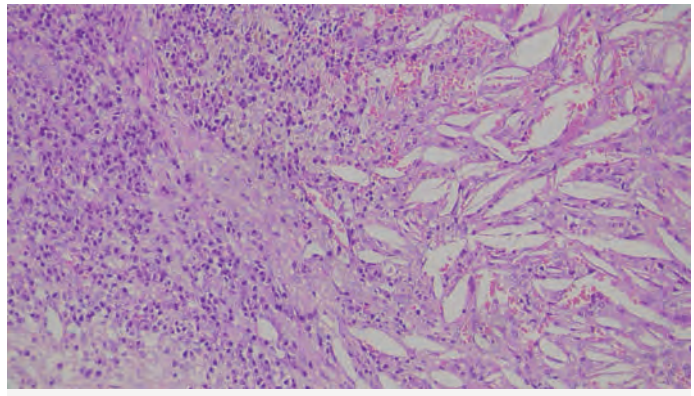

Figure 2. Lymphoplasmacytic inflammation on the left and a foreign body reaction to cholesterol clefts on the right (×40).

DISCUSSION

Middle ear actinomycosis typically presents with recurrent ear discharge resembling chronic suppurative otitis media. A history of acute otitis media may also be present. Mild fever has been reported in approximately 50% of cases, whereas pain is rare. In our case, recurrent ear discharge and ear pain were present, along with a history of acute otitis media. Although hearing loss has been reported in some cases, our patient had no hearing complaints. Because of limited resources, audiometry could not be performed. This is a limitation of our study.

Because otoscopy often fails to provide definitive findings, clinicians may prefer CT imaging. A definitive diagnosis requires isolation and identification of the pathogen by culture or histopathological examination.[5] In our case, the definitive diagnosis was established through histopathological examination. Middle ear swab cultures are generally not helpful for identifying *Actinomyces*, and cultures yield false-negative results in more than 70% of cases.[1] Surgically excised biopsy specimens or purulent material drained from collections are the most appropriate samples for diagnosis.[2] When a surgeon encounters "yellow granules" or unusually inflamed tissue during surgery, an anaerobic culture with prolonged incubation should be requested from the microbiology laboratory.[1]

In a case report by Modi et al.,[4] a 35-year-old patient with a 9-month history of yellow otorrhea was diagnosed with actinomycosis based on biopsy findings. Similarly, Sheikh et al.[6] reported middle ear actinomycosis in a 24-year-old patient with an 8-year history of ear pain and hearing loss. In a study conducted at a tertiary care hospital in Singapore between January 2004 and December 2020, 3 of 10 pediatric patients diagnosed with actinomycosis had ear pain; all but 1 underwent surgical excision, and treatment included penicillin, amoxicillin, or amoxicillin-clavulanate.[7]

Cholesterol granuloma is a foreign body reaction to cholesterol crystals that develops during an inflammatory process, and, as in our case, cholesterol granuloma and *Actinomyces* may sometimes be found together.[8] A study by Erdem showed that *Actinomyces* accompanies cholesterol granuloma in the mandible.[9] Similarly, a study by Maradeix et al.[10] revealed that *Actinomyces* and cholesterol granuloma are found together in the hip. This coexistence may suggest a synergistic relationship between the two conditions. In our case, excision of the polypoid lesion and mastoidectomy promoted aeration and healing by physically removing not only the infected tissue but also the cholesterol granuloma.

Long-term antibiotic treatment should be administered after surgery to achieve disease remission. Depending on disease severity, intravenous penicillin G may be administered for 2 to 6 weeks, followed by oral penicillin V or amoxicillin for 3 to 6 months.[2] Current literature recommends 3 months of treatment for surgically treated patients; however, if the patient has not undergone surgery or has not shown improvement, treatment may be continued for up to 12 months.[1,5] Penicillin V is superior to amoxicillin because of its narrower spectrum of activity, high sensitivity against *Actinomyces*, and the virtually complete absence of resistance. Therefore, penicillin V may be the preferred choice. In patients with penicillin allergy, clindamycin, doxycycline, or fluoroquinolones may be used.[1]

CONCLUSION

Although middle ear actinomycosis is rare, it is a serious infection that is often misdiagnosed or overlooked, primarily because of low culture sensitivity. In our case, a patient with a history of acute otitis media and long-standing ear discharge and pain underwent debridement with a preliminary diagnosis of cholesteatoma and was subsequently diagnosed with actinomycosis on the basis of pathological findings. Middle ear actinomycosis should be considered in patients with chronic otitis that does not respond to treatment.

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from the patient's legal guardians for the publication of this case report and the accompanying images.

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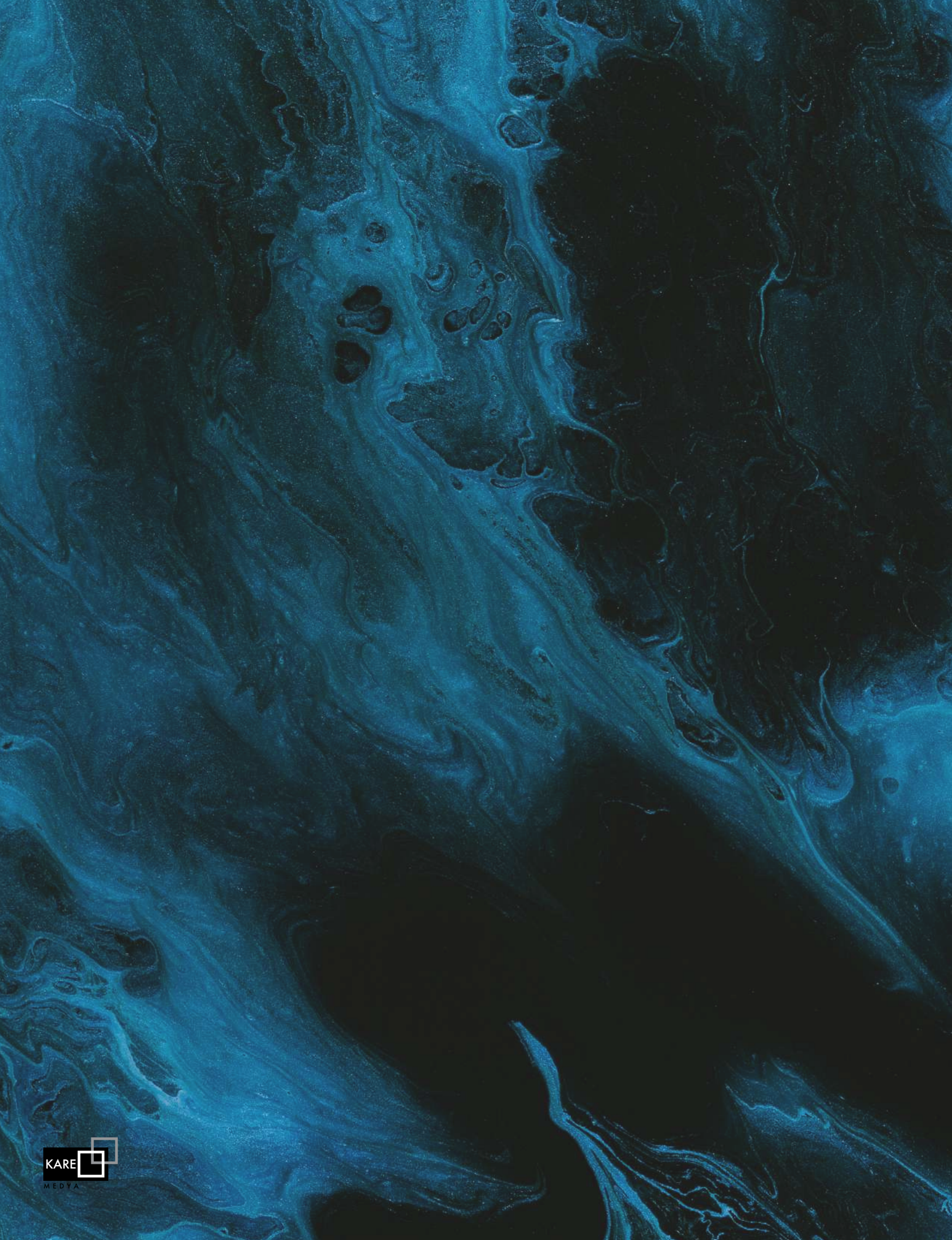
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