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Health misinformation and disinformation: Old narratives, new technologies

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Abstract

This editorial discusses how health misinformation and disinformation combine long-established narratives with new technologies that substantially increase the speed, reach, personalization, and apparent credibility of misleading content. The novelty of the contemporary information environment lies less in entirely new false narratives than in digital infrastructures that allow misleading content to be created, tailored, disseminated, and monetized at unprecedented scale. Online social media platforms and generative artificial intelligence (AI) have transformed these conditions, enabling misleading health information to be created and disseminated at previously impossible scale and speed. Health misinformation and disinformation can influence behavior, undermine trust in scientific and public health institutions, delay appropriate care, promote ineffective or harmful interventions, and weaken responses to disease outbreaks. Their effects may be particularly pronounced among populations with limited access to reliable information, healthcare, and trusted institutions. This editorial also examines how engagement-driven platform design, commercial and political incentives, synthetic media, and inconsistent safeguards amplify the problem. It argues that fact-checking and individual media literacy, while necessary, are insufficient on their own. A more effective response requires timely, transparent public health communication; institutional support for healthcare professionals; greater platform accountability; systematic safety evaluation of generative AI; independent access to relevant data; and policies that address the incentives sustaining the production and circulation of false and misleading health information.

Key words: public health, vaccine hesitancy, disinformation, health misinformation

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Highlights

- Health misinformation and disinformation undermine trust, distort risk perception, delay care, and weaken adherence to evidence-based public health recommendations.
- Social media platforms amplify familiar false narratives through engagement-driven algorithms, personalized recommendations, echo chambers, and coordinated inauthentic activity.
- Generative artificial intelligence (AI) increases the scale, speed, personalization, and apparent credibility of persuasive false health content.
- Effective responses require coordinated action by public health institutions, social media platforms, AI developers, healthcare professionals, and regulators.

Introduction

Misinformation generally refers to false or misleading information shared without the intention to deceive, whereas disinformation involves the deliberate creation or dissemination of false or misleading content intended to manipulate or deceive. In the context of health, neither is a new phenomenon. Long before social media emerged, misleading claims about disease, prevention, and treatment circulated through pamphlets, newspapers, informal networks, commercial advertising, and interpersonal communication. Miracle cures, vaccine scares, conspiracy narratives, and allegations directed at medical institutions have repeatedly accompanied periods of uncertainty and social change.^{1–11} Table 1 summarizes selected historical episodes illustrating the circumstances, recurring themes, driving forces, and public health consequences of medical misinformation and disinformation. What distinguishes the contemporary information environment is less the emergence of entirely new misinformation narratives than the technological systems that enable their production, personalization, amplification, and monetization. Established narratives now circulate across platforms designed for rapid communication and sustained user engagement, while generative artificial intelligence (AI) can substantially increase the scale, speed, and efficiency of creating persuasive false or misleading content.

This convergence makes the spread of health misinformation and disinformation a structural public health concern, not merely a problem of inaccurate communication. A substantial body of evidence indicates that false and misleading health information can undermine trust, distort risk perceptions, delay appropriate care, encourage harmful treatment choices, and weaken adherence to evidence-based recommendations.^{8,12–14} These consequences are especially visible during infectious disease outbreaks, when uncertainty is high, scientific knowledge evolves rapidly, and public cooperation is essential. However, the problem extends far beyond public health emergencies and affects vaccination, cancer treatment, chronic disease management, nutrition, tobacco use, vaping, and the promotion of unproven health products.

Old narratives remain influential

The persistence of health misinformation and disinformation partly reflects the fact that they rarely depend on entirely original ideas. Their recurring narratives are familiar: authorities conceal the truth, scientists are influenced by commercial or political interests, natural remedies are suppressed, a simple cure exists but is withheld for profit, vaccines are more dangerous than the diseases they prevent, and personal testimony is more trustworthy than population-level evidence. Such claims may be persuasive because they offer simple explanations for complex events, identify clear causes or responsible actors, and provide a sense of control in uncertain situations.

These narratives frequently exploit pre-existing mistrust. Personal experience, social identity, scientific and health literacy, institutional performance, political context, and perceived exclusion from decision-making shape responses to health information. Susceptibility to health misinformation has been associated with lower literacy, conspiracy thinking, and greater reliance on social media rather than professional sources of health information.^{15,16} In communities that have experienced discrimination, inadequate access to care, or institutional neglect, distrust may have an understandable historical basis, even when it is subsequently redirected towards unsupported or false explanations. Therefore, addressing misinformation and disinformation requires more than repeatedly instructing the public to trust scientific or governmental authorities. Trust must be developed and maintained through institutional competence, transparency, accountability, and equitable access to healthcare and reliable information.¹⁷

Despite the conceptual distinction between misinformation and disinformation, the boundary between them can become difficult to identify once content begins to circulate. A strategically crafted narrative may be sincerely repeated by individuals who believe it to be true. Consequently, the same claim may originate as disinformation but subsequently spread predominantly as misinformation. Public health responses must consider both the possible origin of false content and the social and technological mechanisms that sustain its circulation.

Table 1. Selected historical episodes of health misinformation and disinformation, illustrating recurrent narratives, their principal drivers and modes of dissemination, and their public health consequences

Main period	Major event	Circumstances of emergence	Primary misinformation theme	Purpose / driving forces	Public health consequences	Reference
1796–1850	Early opposition to smallpox vaccination	Introduction of the first vaccine challenged prevailing religious beliefs and traditional concepts of disease causation.	Vaccine safety, divine intervention, natural immunity.	Religious opposition, fear of novel medical interventions, resistance to scientific innovation.	Reduced vaccine uptake and recurrent smallpox outbreaks.	1
1853–1900	Organized anti-vaccination leagues	Mandatory vaccination legislation stimulated organized political resistance in Europe and North America.	Government overreach, violation of individual liberty.	Political mobilization, protection of individual rights, distrust of authorities.	Declines in vaccine coverage and localized epidemics of preventable diseases.	2
1918–1920	Spanish influenza disinformation	Wartime censorship and limited information created an environment conducive to rumors and false explanations.	False origins of influenza, ineffective remedies, conspiracy narratives.	Political censorship, maintenance of public morale, uncertainty and fear.	Delayed adoption of preventive measures and increased mortality.	3
1950–1970s	Tobacco industry disinformation	Epidemiological evidence linking smoking to disease was deliberately challenged by tobacco industry strategies.	Smoking is safe; doubt about scientific evidence; alternative causation.	Protection of commercial interests through manufactured uncertainty and deception.	Delay in tobacco-control policies and millions of preventable deaths.	4
1974–1985	Whole-cell pertussis (DTP) vaccine controversy	Media amplification of reports alleging neurological injury after vaccination, without causal evidence.	Vaccine safety; link between DTP vaccine and neurological damage.	Public fear, litigation, media sensationalism, erosion of trust.	Reduced vaccination coverage and pertussis resurgence in several countries.	5
1981–1995	HIV/AIDS denialism and disinformation	Scientific uncertainty combined with stigma, political ideologies and misinformation campaigns.	HIV does not cause AIDS; denial of antiretroviral efficacy; conspiracy theories.	Ideological resistance, political agendas, distrust in science and public health institutions.	Delayed diagnosis and treatment, increased HIV transmission and preventable deaths.	6
1998	Wakefield MMR–autism fraud	A fraudulent publication falsely claimed a link between MMR vaccine and autism; later retracted.	Vaccine–autism link.	Scientific misconduct, financial conflict of interest, media amplification.	Long-term decline in vaccine confidence and measles resurgence.	7
2003–2015	Expansion of Internet health disinformation	Web 2.0 and social media enabled rapid, global dissemination of unverified health information.	Alternative medicine; anti-vaccine narratives; miracle cures.	Algorithmic amplification, commercial profit, online communities and echo chambers.	Increased vaccine hesitancy and erosion of trust in health authorities.	8
2014–2016	Ebola disinformation	Distrust in institutions, cultural beliefs and information vacuum during the outbreak facilitated rumors.	False causes of Ebola, traditional cures, conspiracy narratives.	Fear, political instability, low institutional trust, cultural factors.	Reduced healthcare utilization, impaired outbreak control and increased transmission.	9
2020–2023	COVID-19 infodemic	Social media ecosystems facilitated an unprecedented infodemic during the COVID-19 pandemic.	Vaccines, masks, SARS-CoV-2 origin, ineffective treatments, conspiracy theories.	Political polarization, conspiracy theories, financial incentives, algorithmic promotion.	Vaccine hesitancy, inappropriate medication use, reduced adherence to public health measures, and excess mortality.	10
2023–present	AI-generated medical disinformation	Generative artificial intelligence (AI) enables production of highly convincing but inaccurate medical content.	AI-generated false medical advice, deepfake health content.	Automated content generation, influence operations, financial incentives.	Increasing difficulty in distinguishing evidence-based information from fabricated content.	11

From inaccurate beliefs to adverse health outcomes

Health misinformation and disinformation warrant attention because they can influence behavior, not only beliefs. Evidence links exposure to false or misleading health

claims with vaccine hesitancy, rejection of public health recommendations, delayed use of standard care, and interest in ineffective or potentially toxic interventions.^{12,14,18} Vaccination remains one of the most widely discussed examples, as misinformation and disinformation surrounding it have been linked to increased hesitancy, reduced

uptake of immunization and other protective measures, and the persistence or recurrence of vaccine-preventable diseases.¹⁹ The introduction of COVID-19 vaccines showed how public uncertainty about newly introduced vaccine technologies, such as mRNA and adenoviral vector vaccines, can facilitate the spread of misinformation and highlighted the need for proactive, evidence-based communication led by scientific and medical experts.²⁰

The effects of health misinformation and disinformation are not restricted to individual decision-making. Both can also burden health systems by requiring clinicians, public health professionals, and institutions to devote considerable time to correcting false claims, responding to avoidable concerns, and managing the consequences of delayed or inappropriate care. During public health emergencies, misleading information may divert resources, complicate risk communication, interfere with access to services, and increase hostility towards healthcare professionals. In conflict-affected settings, disinformation may interact with propaganda, insecurity, and attacks on healthcare workers or facilities, thereby further weakening already vulnerable systems.^{12,17}

Exposure to and the consequences of health misinformation and disinformation may also reflect existing social inequalities. Populations with limited access to trusted clinicians, high-quality education, reliable digital infrastructure, or stable institutions may be more exposed to misleading content and less able to assess its credibility. Conflict-affected and socioeconomically disadvantaged groups may experience both greater informational vulnerability and more severe consequences when misleading claims affect healthcare-seeking behavior.^{17,21} Therefore, framing the problem exclusively as a deficit in individual

critical thinking may obscure the broader conditions that determine who has access to reliable information and who bears the greatest risk of harm.

Social media and the architecture of amplification

Modern online social media did not create health misinformation or disinformation, but transformed their reach, velocity, and visibility (Fig. 1). False or misleading claims can now be produced and disseminated effectively by non-experts, self-described experts, influencers, commercial actors, ordinary users, and coordinated networks, often with little editorial gatekeeping.^{14,22,23} Systematic reviews consistently identify major social media platforms as key channels for health misinformation and disinformation, particularly around vaccination, infectious disease outbreaks, cancer, and other topics marked by uncertainty or strong emotional responses.^{8,24–26} The problem extends beyond the mere presence of inaccurate material online. Many platforms are primarily optimized for user engagement, and content associated with novelty, fear, anger, certainty, or personal relevance may attract disproportionate attention. Personalized recommendation systems curate information based on past behavior, while social networks may cluster users around shared identities, values, and beliefs. Repeated exposure can increase perceived familiarity and, under some conditions, a claim's apparent credibility. Echo chambers and selective exposure may consequently reinforce the perception that misleading information is widely accepted or socially validated.^{27–29}

These mechanisms may produce an asymmetry between evidence-based communication and misleading content.

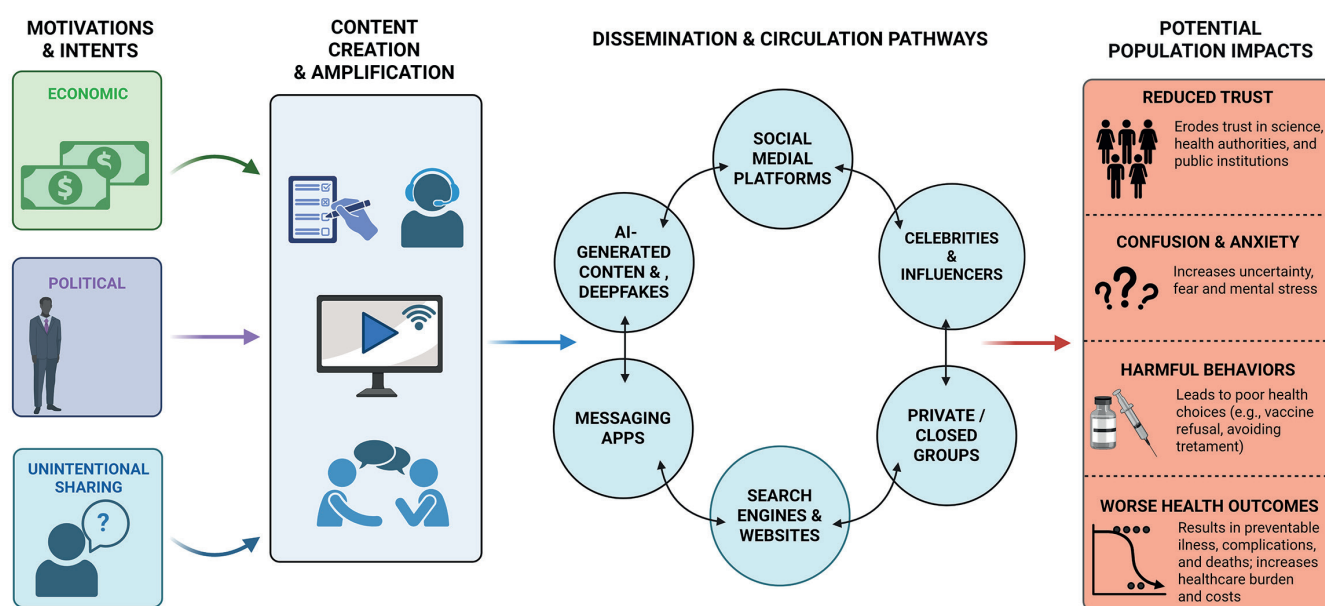


Fig. 1. Conceptual overview of the principal motivations, pathways of dissemination and amplification, and population-level consequences of health disinformation. Contemporary circulation can involve individual, political, and commercial actors and may be amplified by social media algorithms, coordinated activity, and generative artificial intelligence (AI). Created in BioRender. Rzymiski, P. (2026) <https://BioRender.com/lqe7oai>

Reliable health communication is often cautious, conditional, and subject to revision because scientific evidence is probabilistic and evolves over time. Misleading claims can instead be presented with certainty, emotional force, and narrative simplicity. For example, reviews of cancer misinformation indicate that inaccurate posts may receive considerable views and engagement, including when they promote unsupported or potentially harmful interventions.²⁶ Bots, trolls, and coordinated actors may further amplify such material and create an artificial impression of popularity, agreement, or controversy.^{24,30}

Commercial incentives are also relevant. Misleading claims concerning wellness products, supplements, unproven therapies, and conspiratorial media may generate revenue for influencers, advertisers, vendors, and platforms. Therefore, the circulation of false and misleading health information should not always be viewed as an accidental consequence of poor information quality. In some contexts, political, commercial, or reputational incentives strongly support it by rewarding attention and making misleading content profitable.^{14,31}

Generative artificial intelligence and increasing scale

Generative AI introduces an additional dimension to this problem. It does not create the underlying motivations for deception, but it can substantially reduce the resources required to produce misleading content (Fig. 1). Language and image-generation systems can create polished text, synthetic testimonials, apparently professional visual material, and fabricated scientific references within a short period of time. Such content can also be adapted to different languages, cultural contexts, demographic groups, or political identities at relatively low cost.

Studies have shown that publicly accessible AI models can effectively generate targeted disinformation about vaccines, cancer, and vaping, including persuasive personal narratives, false references, and realistic images.^{32,33} These capabilities create a credibility challenge that extends beyond obviously implausible material. Users may interpret linguistic fluency, professional presentation, visual realism, or apparently scientific citations as indicators of reliability, even when the underlying information is inaccurate or fabricated.

Generative AI may also make it increasingly difficult to distinguish authentic materials from synthetic or manipulated content. Deepfake audio or video may falsely attribute statements to clinicians, researchers, or public officials. Synthetic patient narratives can imitate spontaneous personal testimony, while automated accounts can create the appearance of widespread agreement. The combination of generative systems with platform amplification may therefore increase both the volume and apparent social credibility of false health claims.

Current safeguards appear feasible but inconsistent. Comparative evaluations have found that some AI models refuse requests to produce health disinformation, whereas others generate such content with limited resistance. Mechanisms for reporting safety vulnerabilities have also varied in accessibility and responsiveness.^{11,33} These findings suggest that voluntary safeguards alone may be insufficient, particularly if even a minority of widely available systems can be used to generate misleading material on a large scale.

Correction is necessary but insufficient

Correcting inaccurate claims remains an essential component of the response to misinformation and disinformation. Nevertheless, available evidence indicates that corrective interventions have moderate and variable effects depending on their source, timing, format, and audience. A meta-analysis found that attempts to correct health misinformation on social media were effective overall, but not uniformly so, and that expert corrections were more effective than those by non-experts.³⁴ Corrections may also reach users only after a false claim has achieved substantial exposure, and repeatedly restating the original claim can unintentionally increase its familiarity.

An effective strategy should therefore extend beyond reactive fact-checking and recognize education as a central pillar of long-term resilience to health misinformation and disinformation. Prebunking approaches may help users identify common techniques such as false experts, conspiratorial framing, fabricated consensus, emotional manipulation, or misleading presentations of scientific evidence. However, these interventions should form part of a broader educational effort encompassing health, scientific, media, and digital literacy. Education should equip individuals not only to assess the credibility of information sources but also to understand how scientific evidence is generated, reviewed, and revised; why expert conclusions may change as new data emerge; and how uncertainty, probability, and disagreement should be interpreted. Such competencies should be developed through formal education, professional training, and accessible lifelong learning, rather than being expected to emerge only during public health crises. Nevertheless, individual vigilance, even when supported by strong educational foundations, cannot fully compensate for structural features that amplify emotionally engaging or misleading content.

Public health institutions must therefore complement educational measures by improving the quality, accessibility, and timing of communication. Delayed, fragmented, or insufficient communication during periods of uncertainty can create informational gaps that alternative, including misleading, explanations readily fill. Communication should clearly distinguish established knowledge from remaining uncertainties and explain why recommendations may change as evidence develops. It should also

support public understanding rather than merely transmit instructions, helping audiences interpret evidence and place evolving guidance in context. Messages should be adapted to different communities, delivered through trusted intermediaries, and responsive to legitimate concerns rather than dismissive of them. In this way, effective communication can reinforce the educational foundations required for informed decision-making and strengthen public resilience to future waves of health misinformation and disinformation.

The spread of health misinformation and disinformation should increasingly be approached as a problem of information ecosystem governance. First, public health institutions require the capacity to identify emerging narratives, assess their potential health consequences, and distinguish widespread confusion from coordinated manipulation. Such activities should focus on publicly circulating information and avoid stigmatizing private beliefs or legitimate disagreement.

Second, social media platforms should provide greater transparency about recommendation systems, advertising practices, coordinated inauthentic behavior, and the reach of harmful health content. Independent researchers require secure and appropriately governed access to platform data to evaluate the scale of the problem and the effectiveness of potential interventions. Removing individual posts without addressing the systems that repeatedly promote similar material is unlikely to provide a sufficient response.

Third, developers of generative AI should be expected to undertake systematic red-teaming focused specifically on health disinformation, publish meaningful safety evaluations, and establish effective channels for reporting vulnerabilities. Provenance systems, watermarking, and content credentials may help users and institutions identify synthetic media, although none should be considered a complete solution. Safeguards must also be evaluated across languages and cultural settings, rather than predominantly in English, because moderation and fact-checking resources are often weakest in populations that may already face significant informational and health inequalities.^{11,32}

Fourth, healthcare professionals require institutional support. Individual clinicians cannot be expected to counter a rapidly evolving information environment within already constrained consultation time. Training in risk communication, access to rapidly updated explanatory materials, and referral pathways to credible resources should form part of routine public health preparedness. This emphasis on institutional support is consistent with recent arguments that translating evidence into practice requires deliberate structures, education, and shared accountability.³⁵

Finally, policy responses should address the political and commercial incentives that support the production and dissemination of misleading health claims. Where disinformation generates profit, influence, or reputational

benefits, corrective communication alone is unlikely to be sufficient. Advertising regulation, consumer protection, platform accountability, and enforcement against fraudulent health claims should therefore be regarded as relevant public health instruments.³¹

Conclusions

Health misinformation and disinformation can no longer be treated as a series of isolated false claims to be corrected after they spread. They are persistent features of the contemporary information environment, reinforced by technologies and incentives that reward speed, certainty, emotion, and engagement over accuracy. Three priorities should guide the public health response: strengthening population resilience through health, scientific, media, and digital literacy; ensuring timely, transparent, and trustworthy communication supported by institutional preparedness; and establishing meaningful accountability for platforms, AI developers, and commercial actors that facilitate the amplification or monetization of harmful content. Old narratives have acquired new power; public health must now develop responses equal to the systems that amplify them.

Use of AI and AI-assisted technologies

Not applicable.

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Reporting machine learning studies in clinical research: Practical guidelines for transparent and reproducible analysis

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Abstract

The expanding use of machine learning (ML) in clinical research has highlighted persistent methodological weaknesses that undermine the validity and interpretability of reported findings. Common issues include data leakage, inappropriate validation strategies, unstable model performance, and overinterpretation of predictive results. Importantly, strong predictive accuracy does not necessarily translate into clinical utility or biological insight, and poorly validated models may create a misleading impression of robustness.

This editorial proposes a concise set of reporting guidelines to improve transparency, reproducibility, and the critical appraisal of ML-based analyses. The framework emphasizes key elements required for evaluation, including study objectives, data preprocessing, validation design, model development, performance assessment, and interpretation. Additional considerations address domain-specific challenges such as deep learning, missing data, and time-dependent structures.

Rather than limiting methodological flexibility, these guidelines establish a minimal reporting standard that enables readers and reviewers to assess the credibility and generalizability of findings. By promoting clarity and completeness in reporting, they aim to reduce bias, improve reproducibility, and support the responsible integration of ML methods into clinical research.

Key words: machine learning, guidelines, reproducibility of results

Cite as

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Highlights

- Machine learning models in clinical research are frequently affected by methodological flaws that compromise validity.
- Data leakage and inappropriate validation strategies remain major sources of bias.
- Predictive performance does not imply clinical usefulness or biological insight.
- Transparent reporting is essential for reproducibility and critical appraisal.
- These guidelines define a minimal standard for evaluating ML-based analyses in clinical studies.

Introduction

With the increasing availability of clinical data and the growing use of machine learning (ML) methods, the correctness of analytical procedures and transparency of reporting have become critical. Machine learning methods can support prediction, pattern recognition, dimensionality reduction, and exploratory analyses. However, their growing popularity has also exposed recurring methodological weaknesses. Despite the widespread use of ML, numerous studies demonstrate deficiencies including improper validation, data leakage, unstable model performance, insufficient calibration assessment, and over-interpretation of model outputs.^{1,2}

These issues are not merely technical limitations. They directly affect the credibility of findings and their ability to generalize beyond the analyzed dataset. In clinical research, a model that performs well only because of flawed validation or hidden leakage does not offer useful prediction. It represents a methodological mirage: impressive from a distance, but disappearing when exposed to real-world data.

The problem is particularly important because ML outputs are often perceived as objective, sophisticated, or inherently superior to those of simpler statistical models. This perception can be misleading. A complex model can still be poorly validated, unstable, clinically irrelevant, or biologically uninterpretable. Conversely, a simpler model with transparent assumptions and appropriate validation may provide more reliable evidence.

The primary objective of these guidelines is to ensure that ML-based analyses are reported in a manner that allows independent verification and reproducibility. A sufficient standard of reporting requires that readers and reviewers can assess the validity of the analysis and, where necessary, reproduce the analytical process. These guidelines are not intended to replace methodological expertise. Rather, they define a minimal reporting standard required for transparent evaluation of ML-based studies.^{3,4}

The present recommendations should be interpreted as journal-specific methodological guidance and are intended to complement, rather than replace, established reporting guidelines appropriate for the study design (e.g., TRIPOD-AI, PROBAST-AI, CONSORT-AI, or STARD-AI,

where applicable). Authors should follow the relevant reporting framework and its AI/ML-specific extensions whenever appropriate.

Objectives

This editorial aims to define a practical and concise reporting framework for ML-based analyses in clinical and experimental research. The proposed guidelines focus on elements that are essential for critical appraisal: the purpose and scope of the model, data handling and preprocessing, validation strategy, model development, final model reporting, performance evaluation, model interpretation, deep learning-specific issues, missing data, time series, anomaly handling, and reproducibility.^{3,4}

The goal is not to restrict methodological innovation. Instead, it is to ensure that innovation is accompanied by enough transparency for readers to understand what was done, why it was done, and whether the conclusions are justified.

Defining the purpose and scope of the analysis

A fundamental starting point in any ML-based analysis is a clear definition of its purpose. Models may serve different roles, including unsupervised exploration, supervised exploratory analysis, or prediction. These objectives are not interchangeable and should not be treated as such.

For unsupervised methods, authors should clearly describe whether the aim was visualization, clustering, dimensionality reduction, or exploratory pattern detection. Such analyses may be useful for generating hypotheses or describing structure in the data, but their outputs should not be presented as predictive, causal, or directly generalizable findings.

Supervised exploratory analyses may identify associations within a specific dataset. However, dataset-specific associations should not be presented as generalizable discoveries unless appropriate validation supports that interpretation. Variables should only be interpreted as generalizable predictors when supported by adequate validation.

Even then, caution is required, as predictive performance alone does not guarantee stability, clinical relevance, or biological meaning.³

Data handling, preprocessing, and leakage

Data handling and preprocessing are among the most critical and error-prone stages of the ML pipeline. Authors should describe all preprocessing steps in sufficient detail, including transformations, scaling, encoding of categorical variables, imputation, feature selection, anomaly handling, and any other operation applied before modeling.

Improper data handling remains a major source of bias, particularly through data leakage. Data leakage can be defined as the unintended use of information not available at the time of prediction.⁵ It may occur in obvious forms, such as the direct inclusion of the outcome or outcome-derived variables, but also in subtler forms. Outcome leakage may arise when post-baseline variables, future measurements, treatment decisions, or features indirectly related to the outcome are included in the model without appropriate justification.^{1,5}

Leakage is dangerous because it can produce apparently excellent model performance while destroying real-world validity. A model may appear accurate not because it has learned a clinically useful pattern, but because it has been given information that would not be available in the intended prediction setting.

To prevent leakage, all preprocessing must be performed within training data during validation. This includes imputation, scaling, feature selection, anomaly detection, and model tuning. If preprocessing is performed before data splitting or using the entire dataset, information from the validation or test data can influence model development and inflate performance estimates.^{3,4}

The data splitting strategy must also be explicitly described. Authors should specify whether training, validation, and test sets were used, or whether cross-validation, nested validation, bootstrap validation, or external validation was applied. For classification tasks, stratification is recommended, especially when class imbalance is present.

Splitting must respect the structure of the data. Observations from the same unit, such as the same patient, visit series, family, center, hospital, or experimental batch, should not be distributed across training and evaluation datasets if this creates dependency. Overlap of related observations across splits can substantially inflate model performance and give a false impression of generalizability.⁴

Missing data

Handling of missing data is a critical component of ML-based analyses. Missingness is rarely neutral and may reflect underlying clinical processes, disease severity,

workflow decisions, access to care, or measurement practices. Therefore, missing data should be treated as part of the analytical problem rather than a technical nuisance.

Authors should report the extent and pattern of missing data and assess whether missingness may influence the results.³ Where possible, the likely mechanism for missing data should be discussed, including whether the missingness may be missing at random or not at random.

Fixed imputation methods, such as replacing missing values with a constant, should generally be avoided unless clearly justified. Data-driven imputation methods are preferred for missing-at-random data. Multiple imputation should be considered when appropriate, with practical implementations such as multiple imputation by chained equations (MICE) and with attention to the uncertainty introduced by the imputation process.⁶

Imputation must be performed within the training data during validation. Performing imputation on the full dataset before splitting can introduce leakage by allowing information from validation or test data to influence the imputed values. Results should be interpreted with consideration of imputation uncertainty.

Data cleaning and anomaly handling

Data cleaning and anomaly handling should be explicitly documented. Authors should describe anomaly detection methods and justify all data cleaning procedures. This includes decisions about outliers, implausible values, duplicated observations, batch effects, technical artifacts, and biologically extreme observations.

The handling of anomalies should be clearly stated. Authors should specify whether anomalies were removed, transformed, winsorized, imputed, corrected, or retained. These decisions may substantially influence model performance and interpretation.⁷

Anomaly detection procedures must also avoid leakage. If anomalies are identified using the entire dataset before validation, information from evaluation data may influence model development. As with imputation and feature selection, anomaly handling should be embedded within the training process when it affects model development.

Model development and final model reporting

Model development requires transparency in both conceptual and technical aspects. The choice of model should be justified in relation to the research question, dataset size, outcome type, predictor structure, and intended use. Authors should avoid presenting model choice as a purely computational decision detached from the clinical or biological problem.

Hyperparameters and the procedure used to select them must be reported. Feature selection methods should be clearly described, including whether selection was supervised or unsupervised, whether it was performed inside the validation loop, and whether the selected features were carried forward into the final model.

Consistency between feature selection and the final model is important. Feature selection based on one model class and final inference or prediction based on another model class can be problematic unless explicitly justified. For example, selecting variables using one algorithm and then interpreting them in a different final model may create a mismatch between the selection mechanism and the reported results.

Automated model selection procedures should not be used as a black box. If automated selection is applied, the full development procedure should be reported, including candidate models, tuning grids, evaluation criteria, and decision rules. Otherwise, the analytical pathway becomes opaque, and the risk of overfitting is difficult to assess.

A clear distinction must be maintained between model development, hyperparameter tuning, feature selection, validation, and final model reporting. Model evaluation must be based on data not used during model development. Performance metrics derived from the same data used for model training, tuning, or feature selection should not be presented as unbiased estimates of model performance.

Authors should specify whether the final model was refitted on the full dataset after validation. If the final model differs from the model evaluated during validation, this difference must be clearly described. The final set of variables, hyperparameters, preprocessing steps, and modeling procedures should be reported in sufficient detail.

Model evaluation and clinical usefulness

Model performance should be interpreted as an estimate rather than a fixed property. This distinction is essential in clinical datasets, especially when sample sizes are small or moderate. Performance estimates may be highly unstable and sensitive to the chosen data split, resampling strategy, or validation procedure.²

Single train-test splits are particularly vulnerable to instability. A favorable or unfavorable split may substantially affect the model's apparent performance. Therefore, single-split results should be interpreted with caution and, where feasible, supported by repeated resampling, cross-validation, bootstrap validation, nested validation, or external validation.

When class imbalance is present, it must be explicitly reported. Authors should describe its extent and any method used to address it, such as resampling, class weighting, threshold adjustment, or alternative performance metrics.

Accuracy alone is often misleading in imbalanced classification problems and should be interpreted with caution.

Whenever possible, ML models should be compared against simpler baseline models. A complex model is not automatically better because it is more sophisticated. If a simpler model demonstrates comparable performance, superior calibration, or greater interpretability, it should be preferred or presented alongside more complex models, as such information is clinically and scientifically relevant.

Model comparison should be based on common and clinically meaningful metrics. For classification, authors should report metrics such as area under the curve (AUC), area under the precision–recall curve (PR-AUC), sensitivity, specificity, balanced accuracy, and accuracy, with accuracy interpreted with caution when the class distribution is imbalanced. For regression, root mean square error (RMSE), mean absolute deviation (MAD), and other relevant error metrics should be reported.

Calibration should be assessed and reported, particularly when predicted probabilities are clinically meaningful. A model may discriminate well but still produce poorly calibrated risk estimates. When clinical use is implied, decision thresholds must be defined and justified. Thresholds should not be chosen post hoc only to make performance appear favorable. The stability of performance and feature importance should be assessed where feasible. This is especially important when models are developed in small datasets or when many predictors are considered. High predictive performance does not imply clinical usefulness.⁸ A model may perform well statistically but still fail to improve decision-making, workflow, patient outcomes, or biological understanding.

Prediction, explanation, and interpretation

Model interpretation requires particular caution. A key distinction in ML-based analyses is the distinction between prediction and explanation. A model can predict an outcome without revealing why the outcome occurs; predictive accuracy reflects associations captured by the model rather than underlying causal structure. High predictive accuracy does not imply that the model has identified meaningful biological mechanisms.

Feature importance measures, including SHapley Additive exPlanations (SHAP) and similar approaches, should be treated as exploratory interpretation tools. They should not be interpreted as evidence of causal, directional, or mechanistic effects.³ Importance reflects contribution to prediction within a given model and dataset; it does not establish that a feature causes the outcome or represents an independent biological pathway.

This distinction is often blurred in clinical ML studies. Predictive features are sometimes described as risk

factors, mechanisms, therapeutic targets, or biomarkers, despite insufficient evidence. Such interpretations should be avoided unless supported by an appropriate study design, causal framework, external validation, and domain-specific justification.

Interpretable models should be included where possible, particularly when the aim involves clinical understanding or decision support. For black-box models, limitations of interpretability should be clearly stated. Model performance does not validate biological mechanisms. It only indicates that, under the reported validation conditions, the model was able to predict the outcome to a certain extent.

Special considerations for deep learning

Deep learning models require additional transparency because of their flexibility, complexity, and high risk of overfitting.¹ Their use should be clearly justified in relation to dataset size, data type, and problem complexity. In small clinical datasets, deep learning may be inappropriate unless supported by transfer learning, strong regularization, adequate validation, or other safeguards.

Authors should describe the model architecture at a level sufficient for reproducibility. The training procedure should be reported, including data splitting, validation strategy, batch size, number of epochs, optimizer, learning rate, loss function, stopping criteria, and any tuning procedures.

Regularization methods should also be described, including dropout, weight decay, early stopping, data augmentation, or other techniques used to reduce overfitting.¹ Hyperparameter tuning and model selection must be performed without using test data. Final performance should be evaluated on data not used during training or model selection.

For image, signal, or sequence data, preprocessing steps and transformations applied to the input data must be described. This includes resizing, normalization, filtering, segmentation, augmentation, feature extraction, and any manual or automated preprocessing. Deep learning outputs should be interpreted with caution and not treated as evidence of causal relationships or biological mechanisms.

Time series and temporally dependent data

Time series and temporally dependent data require validation strategies that respect the temporal structure of the problem. Authors should describe relevant temporal components, including trends, seasonality, cyclic patterns, and time-related dependencies where applicable.

Random splitting of temporally ordered observations should be avoided unless explicitly justified. Such splitting may allow future information to influence model training and can lead to overly optimistic performance estimates. In prediction problems where the goal is to forecast future outcomes, validation should mimic the intended use case.

Appropriate approaches include temporal holdout validation, rolling-origin evaluation, and walk-forward validation. The key principle is simple: information from the future should not be used to predict earlier observations.

Reproducibility and transparency

Reproducibility is a fundamental requirement for scientific validity. In the context of ML, lack of transparency in preprocessing, model development, tuning, validation, or evaluation effectively prevents independent verification of results. Authors should report the software, programming language, and package versions used in the analysis. Code and preprocessing scripts should be provided where possible. Random seeds should be reported when relevant, although authors should also recognize that setting a seed does not by itself guarantee robustness. Data sources and access conditions should be clearly described. Repository links should be provided for public datasets. If code, data, trained models, or preprocessing scripts cannot be shared, authors should provide a clear reason. Transparent reporting does not require that every study use the same method. It requires that the method used be understandable, evaluable, and, where possible, reproducible.

Conclusions

Machine learning methods can contribute meaningfully to clinical and experimental research, but only when their development, validation, interpretation, and reporting are transparent. Apparent predictive performance is not enough. Without appropriate validation, leakage prevention, calibration assessment, careful handling of missing data, and reproducible reporting, ML models may produce results that are difficult to trust and impossible to reproduce.

These guidelines define a minimal reporting standard for ML-based analyses submitted to the journal. Their purpose is not to discourage methodological innovation, but to ensure that such innovation is accompanied by scientific accountability. Models should be evaluated not only by their complexity or the impressiveness of their performance, but also by whether their results are valid, reproducible, interpretable, and clinically meaningful.

A concise summary of the proposed reporting framework, including minimum reporting requirements, recommended good practices, and common methodological errors, is presented in Table 1.

Table 1. Checklist for reporting machine learning analyses

Reporting element	Minimum requirement	Recommended good practice	Common error
Purpose of analysis	Clearly define the clinical question, intended use, and whether the analysis is exploratory, predictive, supervised, or unsupervised.	Justify the clinical relevance of the task and specify the target population and decision context.	Treating exploratory pattern-finding as generalizable prediction or clinical inference.
Data splitting and validation	Describe the train/test split, cross-validation strategy, or external validation approach.	Use nested cross-validation or external validation where feasible.	Data leakage between training, validation, and test datasets.
Preprocessing	Report all preprocessing, normalization, transformation, and feature engineering procedures.	Perform preprocessing within each validation fold or training split.	Applying preprocessing to the full dataset before splitting.
Data structure	Ensure that splitting respects patient-, visit-, image-, specimen-, batch-, or center-level dependencies.	Assess clustering, batch effects, center effects, and repeated measurements where relevant.	Allowing related observations from the same patient or center to appear in both training and test sets.
Model development	Describe the model type, candidate predictors, feature selection strategy, and hyperparameter tuning.	Compare complex models against simpler baseline or clinically interpretable models.	Automated model selection without transparent reporting of the modeling process.
Model evaluation	Report metrics appropriate to the task, including discrimination, calibration, and clinical usefulness where relevant.	Assess uncertainty, model stability, and stability of selected predictors or feature importance.	Reporting only accuracy, area under the curve (AUC), or a single optimistic performance estimate.
Interpretation	Distinguish prediction, association, causality, and biological interpretation.	Use interpretable models or model explanation tools where appropriate, while acknowledging their limits.	Overinterpreting feature importance as causal or mechanistic evidence.
Class imbalance	Report outcome prevalence and any methods used to address imbalance.	Use appropriate resampling, weighting, threshold analysis, or clinically meaningful performance metrics.	Ignoring imbalance or relying on accuracy in rare-outcome settings.
Missing data	Report the amount, pattern, and handling of missing data.	Use appropriate imputation strategies and sensitivity analyses where feasible.	Fixed ad hoc imputation without reporting or assessing its impact.
Deep learning, where applicable	Describe architecture, input representation, training procedure, validation, and tuning strategy.	Report regularization, stopping criteria, augmentation, and computational reproducibility.	Using test data during architecture selection or hyperparameter tuning.
Time series, where applicable	Use temporally appropriate validation and report the temporal structure of the data.	Assess temporal drift, updating needs, and stability over time.	Random splitting of temporally ordered observations.
Reproducibility	Report software, package versions, random seeds, and data/code availability.	Provide analysis code, model specifications, or repository links when possible.	Insufficient methodological detail preventing replication or critical appraisal.

Use of AI and AI-assisted technologies

ChatGPT (OpenAI) was used to support language editing and drafting of the manuscript text. The authors reviewed, revised, and approved the final content and remain fully responsible for the accuracy, integrity, and interpretation of the manuscript.

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Association of EBV infection with increased cancer aggressiveness in prostate cancer patients: A systematic review and meta-analysis

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Conflict of interest

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Abstract

Background. Several studies have reported the presence of Epstein–Barr virus (EBV) in prostate carcinoma tissues and have investigated how EBV might contribute to prostate cancer (PCa) development. To the best of our knowledge, no comprehensive systematic review and meta-analysis have been performed to: 1) estimate the pooled prevalence of EBV in PCa, particularly in samples with high and low Gleason scores; and 2) examine the association between EBV and PCa aggressiveness, measured using high-grade and/or Gleason scores.

Objectives. This study aimed to achieve 2 primary objectives: 1) to estimate the pooled prevalence of EBV in PCa; and 2) to determine the association between EBV infection and cancer aggressiveness, specifically in relation to moderate-to-high Gleason scores (7 and above).

Materials and methods. PubMed, Embase, and Google Scholar were systematically searched for relevant observational studies on EBV and PCa from database inception through December 31, 2024. The quality of the studies was assessed using the JBI criteria based on study design. A meta-analysis was conducted using 9 and 4 studies, respectively, to estimate the pooled EBV prevalence and the association between EBV infection and Gleason scores, along with 95% confidence and prediction intervals.

Results. A total of 9 studies were included in the analysis. The pooled EBV prevalence among 927 PCa samples was estimated at 33% (95% confidence interval (95% CI): 16–52%; $I^2 = 94.17\%$), while EBV prevalence in PCa samples with moderate-to-high (≥ 7) and low (6) Gleason scores was 59% (95% CI: 21–92%; $I^2 = 90.86\%$) and 34% (95% CI: 15–56%; $I^2 = 0.00\%$), respectively. Similarly, EBV presence in PCa samples was associated with increased odds of moderate-to-high Gleason scores (odds ratio (OR) = 2.38, 95% CI: 1.82–3.13, $I^2 = 0.00\%$, $p = 0.002$).

Conclusions. This study highlights EBV as a possible risk factor for aggressive prostate cancer (PCa). Additional research is necessary to explore how EBV proteins contribute to PCa development.

Key words: prostate cancer, Epstein–Barr virus, Gleason score, cancer aggressiveness

Highlights

- EBV detected in prostate cancer: Meta-analysis shows Epstein–Barr virus (EBV) present in ~33% of prostate cancer (PCa) cases, supporting a potential viral role in tumor biology.
- Higher EBV prevalence in aggressive PCa: EBV was more frequently detected in tumors with Gleason scores ≥ 7 (59%) compared to low-grade tumors (34%).
- Association with tumor aggressiveness: EBV infection was significantly linked to increased odds of moderate-to-high Gleason scores (OR = 2.38), suggesting a role in PCa progression.
- Emerging viral biomarker potential: Findings highlight EBV as a possible risk factor and biomarker for aggressive prostate cancer, warranting further mechanistic and clinical research.

Introduction

Globally, prostate cancer (PCa) ranks as the 2nd most frequently diagnosed cancer and the 5th leading cause of death among men aged 55–65 years.^{1–3} In 2020, it accounted for approx. 43% of all cancers diagnosed in men, alongside lung and colorectal cancers.⁴ Based on GLOBOCAN 2020 data, PCa accounted for about 1.4 million new male cancer cases, representing 15% of all cancers diagnosed in men.⁵

To support prognostic and treatment decisions, the International Society of Urological Pathology (ISUP) and the World Health Organization (WHO) adopted the Gleason grading system, combined with serum prostate-specific antigen (PSA) levels, as the standard method for assessing prostate adenocarcinoma.^{6–8} The Gleason grading system is based on histological tissue patterns, ranging from Gleason 1 to Gleason 5, with Gleason 1 indicating the most differentiated histopathological pattern and a favorable prognosis, while Gleason 5 signifies the least differentiated tumor and a poor prognosis.⁹

A typical Gleason score consists of 2 numbers – the primary and secondary grades – assigned to different regions of the tumor. These 2 numbers are combined to produce a final score between 6 and 10. For example, a score of 3 + 3 = 6 means the tumor is entirely composed of grade 3 features, while a score of 5 + 5 = 10 indicates the tumor is entirely grade 5.⁹

The oncogenesis of PCa can be attributed to various genetic, environmental, and acquired factors. Several viruses have been demonstrated to infect prostate tissue, including cytomegalovirus (CMV), herpes simplex virus 2 (HSV-2), human papillomavirus (HPV), Kaposi's sarcoma-associated herpesvirus (KSHV), and Epstein–Barr virus (EBV), which are notably associated with the progression of PCa.^{10–12}

Several studies have reported varying prevalence rates of EBV in prostate carcinoma tissues and have investigated how EBV might contribute to PCa development. Additionally, analysis of the EBV latency profile has shown that EBV exhibits an “II/III-like” latency pattern in PCa tissues.¹³

Similarly, other studies examining the link between EBV and PCa have found that patients with a history of infectious mononucleosis (caused by EBV) exhibit a significant

trend toward an increased risk of developing PCa.¹⁴ Furthermore, EBV infection- and latency-related gene expression has been associated with the survival of precancerous cells with anti-apoptotic properties, which may promote PCa progression.^{14,15}

One study analyzing PCa tissues with and without EBV revealed significantly higher primary and total Gleason scores in tissues infected with EBV.¹³

Objectives

To the best of our knowledge, no comprehensive systematic review and meta-analysis have been conducted to: 1) estimate the pooled prevalence of EBV in PCa, particularly in samples with high and low Gleason scores; and 2) examine the relationship between EBV infection and PCa aggressiveness, assessed through high-grade and/or Gleason scores. An earlier study by Jafari-Sales et al.¹⁶ examined the presence of EBV in PCa; however, its scope was limited, focusing only on the percentage of EBV-positive PCa samples and the prevalence of EBV in PCa by region. It did not evaluate the relationship between EBV status and clinicopathological parameters, including tumor grade.

In contrast, the present study applies formal meta-analytic methods to quantify the pooled prevalence of EBV in PCa and to evaluate its association with Gleason grade as a marker of tumor aggressiveness.

Methodology

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines for reporting systematic reviews and meta-analyses (Fig. 1).¹⁷ The study used the PICO framework, where the Population (P) was PCa patients, the Exposure (E) was PCa samples with EBV infection, the Comparison (C) was PCa samples without EBV infection, and the Outcome (O) was increased PCa aggressiveness.¹⁸

The study protocol was registered in the PROSPERO database (registration No. CRD42024594565).

Search strategy

For this systematic review and meta-analysis, a comprehensive search was conducted in the PubMed, Embase, and Google Scholar (for gray literature) databases using the keywords “EBV,” “prostate cancer,” and “progression,” or “aggressiveness” (Table 1) to identify articles from database inception through December 31, 2024.

The Google Scholar search was limited to the first 200 results (approx. 20 pages). Additionally, references from the shortlisted articles and the articles citing them were reviewed to identify any missed citations. The search was performed without language restrictions, including articles in all languages and from all regions.

Eligibility criteria and selection of studies

This study included only original observational research (cross-sectional, case-control, and cohort studies) that reported data on PCa, EBV presence or absence, and cancer

aggressiveness (e.g., Gleason scores). Studies lacking any of these data were excluded. Similarly, narrative reviews, systematic reviews, meta-analyses, case reports, and similar publications were also excluded. Articles from PubMed, Embase, and Google Scholar were imported into EndNote X7 (Clarivate Analytics, Philadelphia, USA), where duplicates were removed. The titles and abstracts of the remaining articles were manually screened, and those that did not meet the eligibility criteria were excluded. Full texts were then retrieved, and articles without full text were also excluded (Fig. 1).

The final set of studies was used for data extraction. Two authors (S.H.A. and Z.O.) performed the search and data extraction, resolving disagreements through discussion with a 3rd author (R.B.) and reaching mutual agreement.

Critical appraisal of studies

Since the studies included in our analyses were either cross-sectional or case-control, the Joanna Briggs Institute (JBI) checklist for cross-sectional and case-control

Table 1. Search strategy

Database	Search string
PubMed	(‘epstein-barr virus’ OR ‘ebv’ OR ‘epstein-barr’) AND (‘prostate cancer’ OR ‘prostate cancer’ OR ‘prostatic neoplasms’ OR ‘prostate carcinoma’) AND (‘incidence’ OR ‘severity’ OR ‘progression’ OR ‘prognosis’ OR ‘aggressiveness’) (‘epstein-barr virus’ OR ‘ebv’ OR ‘epstein-barr’) AND (‘prostate cancer’ OR ‘prostate cancer’ OR ‘prostatic neoplasms’ OR ‘prostate carcinoma’) AND (‘incidence’ OR ‘severity’ OR ‘progression’ OR ‘prognosis’ OR ‘aggressiveness’)
Embase	(‘epstein-barr virus’/exp OR ‘epstein-barr virus’ OR ‘ebv’/exp OR ‘ebv’ OR ‘epstein-barr’) AND (‘prostate cancer’/exp OR ‘prostate cancer’ OR ‘prostatic neoplasms’/exp OR ‘prostatic neoplasms’ OR ‘prostate carcinoma’/exp OR ‘prostate carcinoma’) AND (‘incidence’/exp OR incidence OR ‘severity’/exp OR severity OR ‘progression’/exp OR progression OR ‘prognosis’/exp OR prognosis OR ‘aggressiveness’/exp OR aggressiveness)
Google Scholar	(“Epstein-Barr virus” OR EBV) AND (“prostate cancer” OR “prostatic neoplasms” OR “prostate carcinoma”) AND (incidence OR severity OR progression OR prognosis OR aggressiveness)

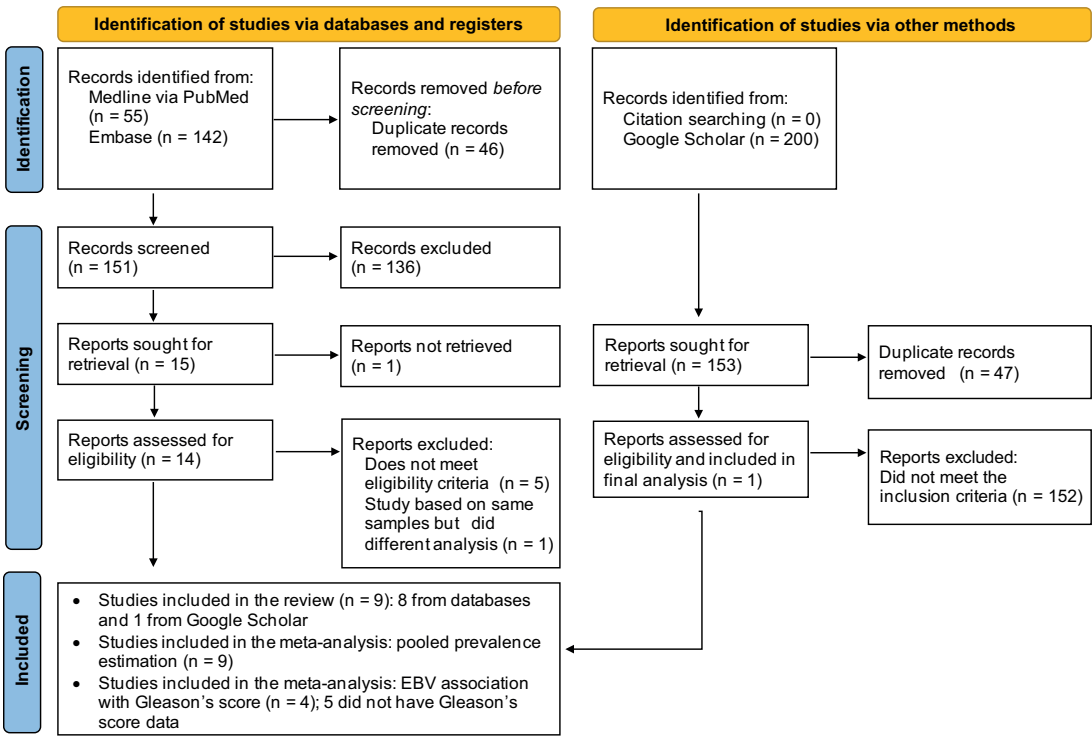


Fig. 1. PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) flowchart showing screening and selection of studies

studies was used to appraise the shortlisted studies.^{19,20} The JBI checklist assesses the methodological quality and risk of bias for each study, using 8 domains/questions for cross-sectional studies and 10 domains/questions for case-control studies. In our analysis, studies scoring above 70% were classified as high-quality, whereas those scoring between 50% and 70% were designated as medium quality.²¹

Data extraction

From the selected studies, data on study authors, study dates, design, location, sample types, total samples (cancer and control), total cancer samples, total control samples, and EBV status in both cancer and control samples (when available) were extracted. Additionally, since Gleason scores were the only marker of cancer aggressiveness reported across all included studies, data on Gleason scores in cancer and control samples, with and without EBV, were also extracted and recorded. The data were then organized in a Microsoft Excel 2013 (Microsoft Corp., Redmond, USA) spreadsheet for further analysis.

Study outcomes

This study aimed to achieve 2 primary outcomes: 1) to estimate the pooled prevalence of EBV in PCa; and 2) to determine the association between EBV infection and cancer aggressiveness, specifically in relation to moderate-to-high Gleason scores (7 and above).

Data synthesis

In this meta-analysis, EBV prevalence was defined as the proportion of PCa samples that tested positive for EBV divided by the total number of PCa samples. The prevalence was expressed as a percentage. All meta-analysis results were presented in a forest plot.

Next, the study estimated the association between EBV status and the presence of: 1) moderate-to-high Gleason scores; and 2) low Gleason scores in PCa tissues. For these analyses, pooled odds were calculated by combining studies with EBV status and Gleason score data. A continuity correction (treatment arm continuity correction) was applied to address studies with zero cells in 2×2 tables.

For all analyses, a random-effects model with restricted maximum likelihood (REML) was used to account for heterogeneity between studies. The 95% confidence interval (95% CI) was estimated using the Knapp–Hartung adjustment for standard errors (SEs). Similarly, the 95% prediction interval was calculated to estimate the range of EBV prevalence or odds ratio (OR) in PCa if new studies are conducted under similar conditions.²²

The OR, 95% CI, and 95% prediction interval were estimated as described above. In all analyses, study heterogeneity was assessed using the tau-squared value (τ^2),

I^2 statistic, and Cochran's Q test. Since the total number of studies in the meta-analyses was fewer than 10, publication bias was not assessed.²³

The subgroup analysis was conducted to estimate the pooled EBV prevalence in PCa tissues: 1) with moderate-to-high Gleason scores; 2) with low Gleason scores; and 3) in normal prostate tissues. To assess the influence of individual studies on the overall results of the meta-analysis, an omit-one-study (OOS)²⁴ sensitivity analysis was performed using the statistical parameters described above. The meta-analysis was performed using Stata v. 19.0 (StataCorp, College Station, USA). In all analyses, $p < 0.05$ was considered statistically significant.

Results

Study selection

A total of 197 records (55 from PubMed and 142 from Embase) were retrieved. Of these, 46 duplicate records were excluded, and the titles and abstracts of 151 studies were further screened.

Of the 151 studies, 14 full-text articles were obtained, while the others were excluded due to irrelevance or unavailability of full texts (Fig. 1). After full-text screening, 6 studies were excluded: 5 did not meet the inclusion criteria, and 1 used the same samples but performed a different analysis. Additionally, a search of gray literature using Google Scholar yielded 200 records, of which 199 were excluded because they were duplicates, did not meet the inclusion criteria, or used the same samples but with different analyses. In total, 9 studies^{25–33} meeting the eligibility criteria were selected for further analysis.

Study characteristics

Of the 9 selected studies, 4 were cross-sectional and 5 were case-control (Table 2).^{25–33} The critical appraisal of cross-sectional studies showed that 3 studies scored 75% and 1 scored 62.5%, indicating moderate to high methodological quality and a potentially low risk of bias (Table 2). Similarly, the appraisal of case-control studies showed that 3 studies were of high quality (score 70%), while 2 were of moderate quality (score 60%), indicating a generally low risk of bias across these studies (Table 2). The publication dates of the 9 selected studies^{25–33} ranged from 2002 to 2024. The studies were conducted in Pakistan, China, the USA, Iraq, Morocco, Iran, Poland, and Australia (Table 3).^{25–33} Four studies included fresh prostate tumor and/or normal samples; 3 used formalin-fixed paraffin-embedded tissue; and 2 only mentioned prostate or normal samples without indicating whether they were fresh or frozen (Table 3). Similarly, 6 studies used polymerase chain reaction (PCR), 2 used immunohistochemistry (IHC), and 1 analyzed RNA sequencing data to detect EBV in PCa

Table 2. Critical appraisal of the shortlisted studies based on the Joanna Briggs Institute (JBI) tool for cross-sectional and case-control studies. The upper and lower sections of the table present cross-sectional and case-control studies, respectively. Q1–Q8 for cross-sectional studies and Q1–Q10 for case-control studies represent the questions used in the JBI tool to assess study quality and risk of bias (see table footnote). A maximum of 1 point can be awarded for each question. The second-to-last (sum) and final (%) columns display the total scores and the overall percentage achieved by each study

Study	Study design	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Sum	%
Whitaker et al. (2013) ²⁵	cross-sectional	1	0	1	1	0	0	1	1	–	–	5	62.5
Mezher and Auda (2017) ²⁶	case-control	0	0	1	1	1	1	0	1	1	0	6	60
Sikora et al. (2024) ²⁷	case-control	1	1	1	1	0	0	0	1	1	1	7	70
Sfanos et al. (2008) ²⁸	cross-sectional	1	1	1	1	0	0	1	1	–	–	6	75
Nahand et al. (2021) ²⁹	case-control	0	1	1	1	1	0	0	1	1	1	7	70
Grinstein et al. 2002) ³⁰	case-control	0	0	1	1	1	0	0	1	1	1	6	60
Chen and Wei (2015) ³¹	cross-sectional	1	1	1	1	0	0	1	1	–	–	6	75
Mharrach et al. (2024) ³²	case-control	0	0	1	1	1	1	0	1	1	1	7	70
Ahmed et al. (2022) ³³	cross-sectional	1	1	1	1	0	0	1	1	–	–	6	75

Questions for cross-sectional studies:

- 1) Were the criteria for inclusion in the sample clearly defined?
- 2) Were the study subjects and the setting described in detail?
- 3) Was the exposure measured in a valid and reliable manner?
- 4) Were objective, standard criteria used to measure the condition?
- 5) Were confounding factors identified?
- 6) Were strategies to address confounding factors stated?
- 7) Were the outcomes measured in a valid and reliable manner?
- 8) Was appropriate statistical analysis used?

Questions for case-control studies:

- 1) Were the groups comparable other than the presence of disease in cases or the absence of disease in controls?
- 2) Were cases and controls appropriately matched?
- 3) Were the same criteria used to identify cases and controls?
- 4) Was exposure measured in a standard, valid, and reliable manner?
- 5) Was exposure measured in the same way for cases and controls?
- 6) Were confounding factors identified?
- 7) Were strategies to address confounding factors stated?
- 8) Were outcomes assessed in a standard, valid, and reliable manner for cases and controls?
- 9) Was the exposure period of interest long enough to be meaningful?
- 10) Was appropriate statistical analysis used?

samples (Table 3). In total, 927 samples were analyzed. Overall, EBV was detected in PCa samples at varying percentages (Table 3). About half of the studies found EBV-positive PCa linked to higher Gleason scores, indicating more aggressive cancer (Table 3).

Epstein–Barr virus prevalence in prostate cancer samples

Epstein–Barr virus prevalence across all prostate cancer samples

Of the 9 studies examining EBV presence in PCa samples, the pooled prevalence was estimated at 33% (95% CI: 16–52%; Fig. 2). Measures of heterogeneity indicated

high variability among studies ($\tau^2 = 0.23$; $I^2 = 94.17\%$; $p < 0.001$). The 95% prediction interval for the overall prevalence ranged from 0% to 88.9%, indicating that future studies are likely to report prevalence estimates within this range.

To evaluate the effect of individual studies, an OOS sensitivity analysis was performed, showing prevalence estimates ranging from 30.9% to 38.6%. The overlapping 95% CIs indicated that no individual study significantly influenced the pooled prevalence (Supplementary Table 1). Publication bias could not be assessed because fewer than 10 studies were included in the meta-analysis. To investigate potential sources of heterogeneity ($I^2 > 75\%$), we conducted several prespecified subgroup analyses based on clinical characteristics and methodological factors. Studies were categorized by geographic

Table 3. Characteristics of the studies included in the analysis

Study	Study design	Location	Sample type	Demo-graphic information	Tumor grade and tumor stage	Total samples (cancer and control)	Total cancer	Total control	Detection method	EBV in cancer	EBV in control	Key findings	EBI score [%]
Whitaker et al. (2013) ²⁵	cross-sectional	Australia	fresh frozen tissues	NR	NR	50	50	NR	PCR	26	NR	High prevalence of HPV and EBV in PCa benign and normal tissues.	62.5
Mezher and Auda (2017) ²⁶	case-control	Iraq	FFPE	age range: 50–90 years	GG: 1 (6), 2 (4), 3 (4), 4 (2), 5 (4)	70	60	10	IHC	7	0	Higher prevalence of HPV and EBV in malignant prostate tissues compared to benign. Prostate cancer samples with EBV were associated with age and Gleason Grade.	60
Sikora et al. (2024) ²⁷	case-control	Poland	fresh frozen tissues	age range: 54–82 years EBV POS (57) vs EBV NEG (58)	EBV POS vs EBV NEG GS: 6 (20 vs 34), 7 (13 vs 16), 8 (11 vs 2), 9 (13 vs 6) T1 (21 vs 33), T2 (36 vs 25), N0 (57 vs 58), M0 (57 vs 68)	155	115	40	PCR	57	NR	EBV was detected in almost half of the PCa samples. The presence of EBV was associated with higher TLR-2 and TLR-9 levels in patients with PCa.	70
Sfanos et al. (2008) ²⁸	cross-sectional	USA	fresh tissues	median age (range): 57 (39–72) years	highest GS: 5 (1), 6 (22), 7 (3), 8 (2), 9 (2) TNM: T2 (24), T3 (6)	200	200	NR	PCR	10	NR	EBV DNA was present in 5% of PCa samples.	75
Nahand et al. (2021) ²⁹	case-control	Iran	prostate tumor and normal samples	age: 52.7 years (PCa) vs 55.6 years (control)	cancer stage: I (4), II (23), III (18), IV (22)	107	67	40	PCR	33	16	HPV/EBV coinfection increased the expression of inflammatory factors and anti-apoptotic mediators, suggesting its role in PCa progression.	70
Grinstein et al. (2002) ³⁰	case-control	USA	FFPE	NR	all grades represented (details not given)	19	19	10	IHC	7	0	EBV was detected in various epithelial carcinomas, including PCa tissues.	60
Chen and Wei (2015) ³¹	cross-sectional	China	prostate tumor and normal samples	NR	GS: 4–8	28	14	14	RNA-seq	1	2	EBV reads were found in some Chinese samples. More PCa samples without EBV belonged to Gleason score groups.	75
Mharrah et al. (2024) ³²	case-control	Morocco	fresh prostate biopsies	age: <60 years (13); >60 years (37)	GS: <6 (14); >6 (36) T-stage: T1 (17), T2 (29), T3 (1), T4 (3)	100	50	50	PCR	32	3	Higher prevalence of EBV in tissue (64%) compared to blood (38%); a significant association between EBV infection and Gleason score ($p = 0.041$) and T-stage ($p = 0.020$)	70
Ahmed et al. (2022) ³³	cross-sectional	Pakistan	FFPE	NR	EBV POS (GS: 8.24) vs EBV NEG (GS: 7.7)	99	99	NR	PCR	39	NR	EBV maintains "II/III-like" latency profile in PCa tissue. The PCa tissues with EBV, compared to those without EBV, had higher perineural invasion and total Gleason scores.	75

EBV – Epstein–Barr virus; EBV POS – EBV-positive samples; EBV NEG – EBV-negative samples; PCR – polymerase chain reaction; IHC – immunohistochemistry; RNA-seq – RNA sequencing; GG – Gleason grade; FFPE – formalin-fixed paraffin-embedded tissue; GS – Gleason score; TNM – tumor–node–metastasis cancer staging system; T1–T4 – tumor stages I–IV; NR – not reported; TLR – toll-like receptor; PCa – prostate cancer.

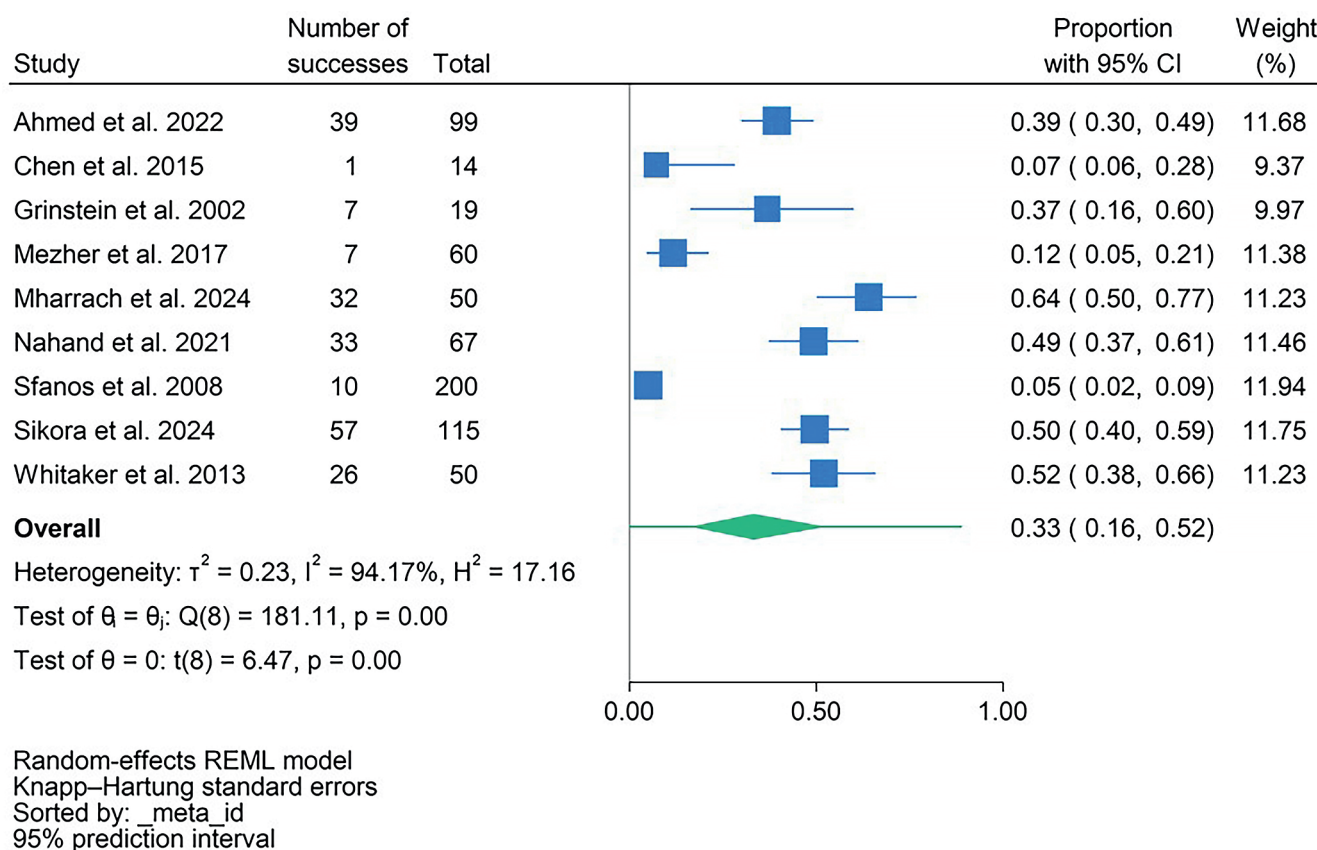


Fig. 2. Forest plot showing pooled Epstein–Barr virus (EBV) in prostate cancer (PCa) samples. The figure shows the number of PCa samples with EBV (number of successes), the total sample, the proportion of EBV with a 95% confidence interval (95% CI) for each study, and the weight of each study contributed to the overall analysis. Blue squares and lines also show the proportion and 95% CI. The overall proportion and 95% CI are indicated with a green diamond and a green line, respectively. The figure also shows values for the heterogeneity tests and details of the parameters used to perform the meta-analysis

region (Western, Middle East/Africa, or East Asia), detection method (PCR, IHC, RNA-Seq), tumor grade (high Gleason grade and mixed/low grade), sample type (fresh, frozen vs formalin-fixed and paraffin-embedded (FFPE) and study design (case-control and cross-sectional). The between-group variance was assessed using Cochran's Q test (Q_b), with a $p < 0.050$ indicating a statistically significant difference between subgroups (Table 4). Subgroup analysis showed that geographic region was a significant driver of this variation ($p = 0.013$), with the highest prevalence observed in the Middle East and Africa (40.7%; $I^2 = 94.64\%$) and the lowest in East Asia (7%). Furthermore, the detection method significantly influenced the reported prevalence ($p = 0.040$), with PCR-based studies reporting a much higher pooled estimate (42.6%; $I^2 = 95.56\%$) than IHC or RNA-Seq. Notably, heterogeneity decreased to 77.97% within the IHC subgroup. However, the estimates should be interpreted with caution due to the small number of studies in each analysis. No significant differences were observed when subgrouping by Gleason grade ($p = 0.456$), sample type ($p = 0.090$), or study design ($p = 0.265$). The OOS sensitivity analysis showed that the pooled prevalence estimates were not influenced by any individual study across the subgroups (Supplementary Table 2).

Epstein–Barr virus prevalence in prostate cancer samples with moderate-to-high (7 and above) and low (6) Gleason scores

This subgroup analysis was conducted using 5 and 4 studies, respectively, as the remaining studies lacked Gleason score data. The pooled EBV prevalence in PCa samples with moderate-to-high (7 and above) Gleason scores was estimated at 59% (95% CI: 21–92%; Supplementary Fig. 1). The heterogeneity measures indicated substantial inter-study variability ($\tau^2 = 0.28$; $I^2 = 90.86\%$; $Q = 0.000$). The 95% prediction interval for pooled prevalence ranged from 0% to 100%. The OOS sensitivity analysis indicated that EBV prevalence ranged from 49.2% to 64.1% in PCa samples with moderate-to-high Gleason scores, with overlapping 95% CIs (Supplementary Table 4), suggesting that no individual study disproportionately influenced the pooled EBV prevalence. A subgroup analysis was performed due to high heterogeneity in the pooled prevalence across samples with moderate-to-high (7 or above) Gleason scores. The studies were categorized based on geographic region (Western, Middle East/Africa, or East Asia), detection method (PCR vs IHC/RNA-Seq), tumor grade (high Gleason grade/associated vs mixed/low grade), sample type (fresh/frozen vs FFPE), and study design (case-control vs cross-sectional).

Table 4. Subgroup analyses to identify the source of heterogeneity in the pooled prevalence estimates

Subgroup category		Number of studies	Pooled prevalence (95% CI)	I ² [%]	p-value (Qb)
Geographic region	Western	4	35.3% (12.7–57.8%)	97.55	0.013*
	ME/Africa	4	40.7% (18.9–62.4%)	94.64	
	East Asia	1	7.0% (0.00–20.6%)	–	
Detection method	PCR	6	42.6% (25.7–59.4%)	95.56	0.040*
	IHC	2	15% (7.0–22.0%)	77.97	
	RNA-Seq	1	7% (1.0–31.0%)	–	
Gleason grade	low/NR	5	29.5% (9.0–49.9%)	94.45	0.456
	high grade/association	4	40.8% (19.2–62.5%)	94.91	
Sample type	fresh	4	31% (1.0–61.0%)	97.40	0.090
	frozen	2	50% (43.0–58.0%)	0.00	
	FFPE	3	29% (7.0–50.0%)	87.15	
Study design	cross-sectional	4	25.6% (2.7–48.4%)	96.18	0.265
	case-control	5	42.1% (24.1–60.1%)	91.77	

ME – Middle East; PCR – polymerase chain reaction; IHC – immunohistochemistry; RNA-seq – RNA sequencing; FFPE – formalin-fixed paraffin-embedded tissue.

(Supplementary Table 3). The analysis indicated possible differences in EBV prevalence across geographic regions ($p = 0.030$), with a pooled prevalence of 46% ($I^2 = 94.2\%$) in the Middle East and Africa. Sample type also significantly influenced prevalence ($p < 0.001$), with FFPE showing a pooled prevalence of 35% ($I^2 = 97.12\%$). No significant differences were observed by detection method ($p = 0.120$).

These results should be interpreted cautiously because many subgroups included only a single study, which does not constitute a true pooled estimate, and heterogeneity was high in some subgroups (I^2 up to 97%). Conversely, the difference attributable to study design was more robust ($p < 0.001$), with case-control studies showing a much higher prevalence (64%; $I^2 = 80.52\%$) than cross-sectional studies (34%; $I^2 = 0.00\%$).

Similarly, the pooled EBV prevalence in PCa samples with low (6) Gleason scores was estimated at 34% (95% CI: 15–56%; Supplementary Fig. 1), with no evidence of inter-study heterogeneity ($\tau^2 = 0.00$; $I^2 = 0.00\%$; $Q = 0.26$). The 95% prediction interval for pooled prevalence ranged from 9.2% to 63.8%. It is important to note that, since only 4 studies were included in this meta-analysis, publication bias could not be assessed.

Epstein–Barr virus prevalence in normal prostate samples

This subgroup analysis was conducted using 5 studies that analyzed the presence of EBV in normal prostate samples. The pooled EBV prevalence was estimated at 17% (95% CI: 6–39%; Supplementary Fig. 1), with low inter-study heterogeneity ($\tau^2 = 0.27$; $I^2 = 37.08\%$; $Q = 0.17$). The 95% prediction interval for pooled prevalence ranged from 0% to 88.9%. It is important to note that, since only 5 studies were included in this meta-analysis, publication bias could not be assessed.

Association of EBV with prostate cancer aggressiveness

Next, the association between EBV and PCa aggressiveness was examined. Only 4 studies were included in this analysis, as the study by Mezher and Auda²⁶ lacked data on Gleason scores in EBV-negative PCa samples.

The results suggested that the presence of EBV in PCa samples was associated with higher odds of moderate-to-high Gleason scores (summary OR: 2.38, 95% CI: 1.82–3.13; $p = 0.002$; Fig. 3). Measures of heterogeneity did not indicate inter-study variability ($\tau^2 = 0.00$; $I^2 = 0.00\%$; $Q = 0.97$), likely due to the limited number of studies. The 95% prediction interval for the overall OR ranged from 1.65 to 3.44.

Additionally, the association between EBV and low Gleason scores was examined. The results indicated that EBV presence in PCa samples was associated with lower odds of low (6) Gleason scores (summary OR: 0.42, 95% CI: 0.32–0.55; $p = 0.002$; Supplementary Fig. 2). Heterogeneity measures did not suggest inter-study variability ($\tau^2 = 0.00$; $I^2 = 0.00\%$; $Q = 0.97$). The 95% prediction interval for the pooled OR ranged from 0.29 to 0.61. It is important to note that, since only 4 studies were included in this meta-analysis, publication bias could not be assessed.

Discussion

This systematic review and meta-analysis aimed to estimate the pooled prevalence of EBV in PCa tissues and to explore the potential link between EBV infection and PCa aggressiveness. Epstein–Barr virus has previously been associated with several cancers, most notably nasopharyngeal and gastric carcinomas,³⁴ where chronic EBV infection, together with host and environmental

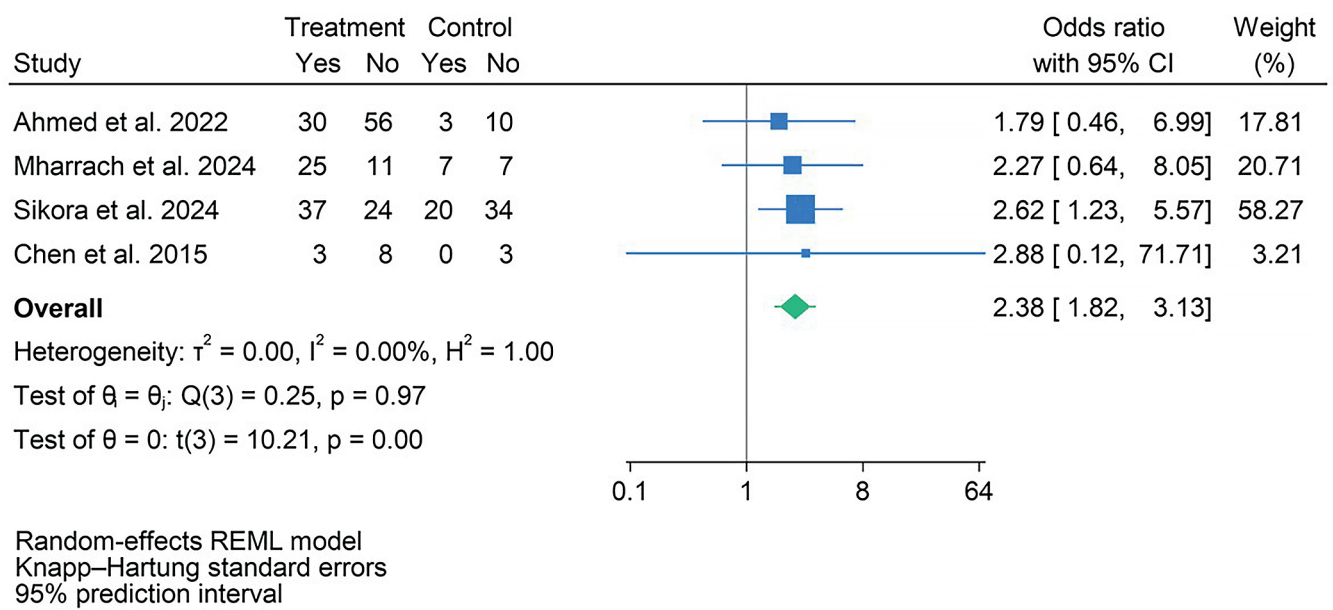


Fig. 3. Forest plot showing odds of moderate-to-high Gleason and Epstein–Barr virus (EBV) status of the prostate cancer (PCa) samples. The figure shows the number of PCa samples with moderate to high Gleason's score (7 or higher) and EBV-positive or -negative status, the odds ratios (ORs) with 95% confidence intervals (95% CIs) for each study, and the weight of each study in the overall analysis. Blue squares and lines also show the ORs and 95% CI. The summary (overall) OR and 95% CI are indicated with green diamonds and lines, respectively. The figure also shows the results of the heterogeneity tests and the details of the parameters used in the meta-analysis

factors, contributes to cancer progression.^{35,36} Within this broader oncogenic context, the present findings provide quantitative evidence that EBV is commonly identified in PCa tissues, thereby emphasizing the need for further research into its role in prostate tumor biology and disease progression.

Our analysis estimated a pooled prevalence of EBV in PCa tissue at 33% (95% CI: 16–52%). Substantial heterogeneity was observed ($I^2 = 94.1\%$), indicating considerable variation across studies, which may be attributed to differences in study location, detection methods, sample type, or study precision.³⁷ However, sensitivity analysis (OOS) yielded pooled prevalence estimates within a narrow range (30–39%), with overlapping 95% CIs, suggesting that no individual study significantly influenced the overall estimate.^{26,33}

Although PCa is not typically considered to be driven by EBV, the grade-dependent enrichment observed in this study is consistent with patterns reported in other EBV-associated epithelial cancers. The EBV–PCa relationship remains poorly characterized. Unlike in lymphoid and other epithelial cancers, studies have reported a wide range of EBV prevalence in PCa tissues (8–64%),^{25–33} with only 1 study noting a higher prevalence of EBV in prostate tissue than in matched peripheral blood samples from the same patients.³² One important yet unresolved question in this field is how EBV infects PCa tissues. Several mechanisms have been proposed for EBV entry into epithelial cells. Current evidence supports a model where viral entry occurs via fusion of the viral envelope with the host cell membrane, mediated primarily by EBV glycoproteins gB and the gH/gL complex.³⁸ In epithelial cells,

gH/gL interacts with integrins such as $\alpha v\beta 5$, $\alpha v\beta 6$, and $\alpha v\beta 8$, facilitating membrane fusion and viral entry.³⁹ Given that prostate epithelial cells express integrins, including $\alpha v\beta 6$ and $\alpha v\beta 8$, particularly in malignant or inflammatory states, it is biologically plausible that these cells are permissive to EBV entry.⁴⁰

In established EBV-associated malignancies, EBV positivity is consistently associated with higher-grade tumors, altered differentiation patterns, and adverse pathological features, suggesting a relationship between viral presence and aggressive disease phenotypes.⁴¹ In PCa, tumor aggressiveness is commonly assessed using the Gleason grading system.⁴²

Our analysis showed a higher pooled prevalence of EBV in PCa tissues with moderate-to-high Gleason scores (59%, 95% CI: 21–92%) compared to low Gleason score tumors (34%, 95% CI: 15–56%) and normal prostate samples (17%, 95% CI: 6–39%). Our analysis further demonstrated that EBV detection rates increase across tumor grades, supporting a grade-dependent relationship rather than a uniform distribution of viral presence across PCa subtypes.

Among the studies included in the meta-analysis, higher EBV viral loads and antibody titers were reported in patients with higher Gleason scores,^{28,29} providing additional support for the pattern identified in our analysis. These findings, combined with the pooled ORs from our study, suggest that EBV detection in PCa may be linked to more aggressive tumors rather than representing incidental background infection.^{28,29}

In EBV-related cancers, such as nasopharyngeal carcinoma, viral persistence is associated with aggressive tumor behavior.⁴¹ However, these findings should be interpreted

with caution because the pooled OR was based on unadjusted estimates, as adjusted ORs were inconsistently reported. The observed association may also be influenced by confounders such as small sample sizes, differences in EBV detection methods, and variability in diagnostic criteria.

Emerging molecular evidence provides a plausible biological explanation for the observed EBV enrichment in higher-grade PCa, as identified in this meta-analysis. A study examining EBV latency in PCa tissue identified a latency II/III-like profile,³³ consistent with expression patterns associated with oncogenic activity in other EBV-related epithelial malignancies.

Additionally, 1 study demonstrated that EBV-positive PCa tissues exhibit higher expression levels of vimentin, a key marker of epithelial–mesenchymal transition, altered expression of several oncomiRs (including miR-146b, miR-183-3p, miR-196a, and miR-126) and oncogenes (such as AR, CDH1, and CDC20), as well as reduced CD3⁺ lymphocytic infiltration, implying a more aggressive and immunosuppressive tumor microenvironment.¹³ Furthermore, a clinical study found that patients with EBV-positive PCa exhibited higher EBV antibody levels and viral loads, which correlated with higher Gleason scores.⁴³ Experimental evidence from prostate and other epithelial tissues suggests that EBV latent gene expression may support the survival of premalignant cells, thereby promoting genomic instability and disease progression.^{44,45}

EBV/HPV co-infection has also been shown to contribute to PCa development by increasing the expression of anti-apoptotic molecules such as survivin and Bcl-2, inflammatory mediators including nuclear factor kappa B (NF- κ B), tumor necrosis factor alpha (TNF- α), interleukin (IL)-6, IL-17, reactive oxygen species (ROS), and anti-apoptosis factors such as N-cadherin and Twist.^{25,46} The EBV/HPV co-infection has further been shown to promote chronic inflammation and facilitate immune evasion, creating a microenvironment that supports cell proliferation, angiogenesis, metastasis, and resistance to apoptosis.²⁹

Taken together, it is biologically plausible that the expression of EBV latent proteins in prostate tissue could contribute to tumor progression and higher Gleason grades,^{27,31–33} although direct evidence supporting this mechanism in PCa remains limited.

Limitations of the study

We acknowledge limitations regarding the generalizability and quality of evidence in our analysis. First, this meta-analysis considered only the Gleason score as an indicator of PCa progression. While the Gleason score is a significant marker, other factors, such as PSA levels and dysregulation of tumor suppressors or oncogenes, also influence tumor aggressiveness. However, since most existing studies focused solely on the Gleason score, we used it as the primary marker. Second, this study included only 9 and 4 studies, which may limit the generalizability

and statistical robustness of the findings and increase uncertainty around the pooled results. Additionally, because the meta-analysis included <10 studies, publication bias could not be assessed.²³ Due to the limited number of published studies in this area, the results may also be affected by publication bias arising from the geographical clustering of the papers,^{47,48} which could result in an over-representation of data from specific regions, reflecting local epidemiological trends, healthcare access, and diagnostic methods rather than providing a global view. This ultimately restricts the applicability of the findings and highlights the need for larger, multi-regional studies to confirm EBV infection in PCa across diverse populations. The presence of confounders, discussed above, may affect the reliability of the analysis and increase the risk of bias and study heterogeneity. Therefore, the findings should be interpreted with caution.

Conclusions

This study provides support for a potential link between EBV infection and PCa aggressiveness and highlights the need for further research into underlying mechanisms. Future research should focus on the following key areas: 1) conducting statistically robust epidemiological studies with large sample sizes to determine the prevalence of EBV in PCa tissues and its association with tumor aggressiveness; 2) investigating the localization of EBV within PCa tissues; 3) examining how EBV genes and proteins, particularly latency-associated proteins, influence tumor progression; and 4) exploring the potential of EBV infection as a prognostic biomarker for aggressive PCa. A deeper understanding of these mechanisms may improve our knowledge of the complex processes underlying prostate carcinogenesis and may also have therapeutic implications.

Supplementary data

The supplementary files are available at <https://doi.org/10.5281/zenodo.19183381>. The package contains the following files:

Supplementary Table 1. OOS sensitivity analysis assessing the effect of omitting each included study on pooled EBV prevalence. Each row presents the omitted study, the proportion, the 95% CI, and the heterogeneity measure (I^2) for EBV in PCa tissues after exclusion.

Supplementary Table 2. OOS sensitivity analysis assessing the effect of omitting each included study on pooled EBV prevalence across subgroups. Each row presents the omitted study, the proportion, and the 95% CI for EBV in PCa tissues after exclusion.

Supplementary Table 3. Subgroups based on high Gleason grade (Gleason 7 and above).

Supplementary Table 4. OOS sensitivity analysis assessing the effect of omitting each included study on pooled

EBV prevalence in PCa samples with moderate-to-high Gleason scores (7 and above). Each row presents the omitted study, the proportion, and the 95% CI for EBV in PCa tissues after exclusion.

Supplementary Fig. 1. Forest plots of pooled EBV prevalence in prostate tissue, stratified by Gleason grade and normal controls. A. PCa with moderate-to-high Gleason scores (≥ 7); B. PCa with low Gleason scores (≤ 6); C. normal prostate samples. For each study, the number of EBV-positive samples, total sample size, and study-specific prevalence with 95% CIs are shown. Study weights are indicated by square size, and horizontal lines represent 95% CIs. The pooled prevalence estimate is shown as a diamond. Heterogeneity statistics and meta-analytic parameters are reported within each panel.

Supplementary Fig. 2. Forest plot showing the association between low Gleason score and EBV status in PCa samples. The figure displays the number of PCa samples with low (6) Gleason scores, their EBV-positive and EBV-negative status, the ORs with 95% CIs for each study, and the contribution weight of each study to the overall analysis. Blue squares and lines represent the ORs and their 95% CIs. The overall (summary) OR and 95% CI are indicated by green diamonds and lines, respectively. The figure also includes heterogeneity statistics and details of the meta-analytic parameters.

Use of AI and AI-assisted technologies

Not applicable.

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Efficacy of continuous ibrutinib monotherapy in patients with relapsed/refractory chronic lymphocytic leukemia with high-burden and low-burden *TP53* mutations: Multicenter retrospective analysis of the Polish Adult Leukemia Group (PALG)

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Abstract

Background. *TP53* gene aberrations, including deletions (del(17p)) and mutations, are the strongest predictors of shorter response duration and survival with most therapies for chronic lymphocytic leukemia (CLL). However, the impact of isolated *TP53* mutations (without concomitant deletion of the 2nd allele) on outcomes of treatment with Bruton tyrosine kinase inhibitors remains less clear, especially in the context of low-burden mutations.

Objectives. This retrospective analysis aimed to assess the efficacy of ibrutinib in patients with relapsed/refractory CLL and *TP53* mutations.

Materials and methods. Patients were selected from a large observational study conducted by the Polish Adult Leukemia Group (PALG) based on the following criteria: the presence of a low- or high-burden *TP53* mutation detected by next-generation sequencing (NGS), the absence of del(17p), and treatment with ibrutinib.

Results. Of the 468 patients tested using NGS, isolated *TP53* mutations were identified in 76 patients, 25 of whom received ibrutinib. This cohort included 13 patients with high-burden mutations (variant allele frequency (VAF) $\geq 10\%$) and 12 with low-burden mutations (VAF $< 10\%$). After a median follow-up of 19 months (range: 1–40 months), the best overall response rate (ORR) in the entire study cohort was 84%, with a median progression-free survival (PFS) of 21 months and a median overall survival (OS) of 21 months. There were no statistically significant differences in PFS or OS between the high- and low-burden groups (hazard ratio (HR) = 0.74 (95% confidence interval (95% CI): 0.28–2.01); $p = 0.559$ and HR = 0.75 (95% CI: 0.28–2.01), respectively).

Conclusions. In patients with isolated *TP53* mutations, the efficacy of ibrutinib did not appear to differ according to mutational burden.

Key words: chronic lymphocytic leukemia, ibrutinib, next-generation sequencing, *TP53* mutations

Highlights

- Ibrutinib is effective in relapsed/refractory chronic lymphocytic leukemia (CLL) with isolated *TP53* mutations. Patients with *TP53* mutations without del(17p) achieved a high overall response rate (ORR) of 84%, supporting BTK inhibitor use in this high-risk genetic subgroup.
- *TP53* mutational burden does not affect ibrutinib outcomes. Progression-free survival (PFS) and overall survival (OR) were comparable between patients with low-burden (variant allele frequency (VAF) $< 10\%$) and high-burden (VAF $\geq 10\%$) *TP53* mutations.
- Next-generation sequencing (NGS) enables refined *TP53* risk stratification. NGS-based detection of low- and high-burden *TP53* mutations helps identify patients who may still benefit from targeted therapy despite adverse molecular features.
- BTK inhibition may overcome *TP53*-associated treatment resistance. These findings suggest that ibrutinib efficacy is preserved regardless of *TP53* mutation load, reinforcing its role as a key therapeutic option in *TP53*-mutated CLL.

Background

Chronic lymphocytic leukemia (CLL) is characterized by a highly heterogeneous clinical course, ranging from indolent disease with prolonged survival without treatment to aggressive forms with rapid progression and poor prognosis.^{1–3} This variability is partly attributed to genetic abnormalities in CLL cells, most notably disruptions of the *TP53* gene through deletion of the chromosome 17p13.1 region (del(17p)), leading to loss of one *TP53* allele, and/or mutations within the *TP53* coding region.

TP53 aberrations are the strongest prognostic and predictive markers in CLL and are associated with poor treatment response and markedly shorter survival in patients treated with chemoimmunotherapy (CIT).^{4–6} Recent advances in CLL therapy have markedly improved outcomes, offering patients with *TP53* abnormalities access to effective targeted agents, B-cell receptor (BCR) and BCL2 inhibitors.^{7–11} Therefore, assessment of *TP53* abnormalities significantly influences therapeutic decision-making in CLL.^{12–14}

Historically, *TP53* mutations in CLL were primarily investigated using conventional Sanger sequencing, which has a detection sensitivity limited to mutations with a variant allele frequency (VAF) of at least 10%. Currently, next-generation sequencing (NGS) is increasingly employed, enabling the detection of low-burden *TP53* mutations, even at a VAF below 1%. Studies have shown that among isolated *TP53* mutations, low-burden mutations are more common.^{15–17} According to the recently updated guidelines from the *TP53* network of ERIC (European Research Initiative on CLL), a fixed VAF threshold is no longer recommended for reporting *TP53* mutations. It remains essential to thoroughly validate or verify testing methods, particularly when identifying mutations present at low allele frequencies.¹⁵ Reporting all *TP53* variants above the laboratory-defined detection limit is recommended. The preferred method is NGS, but Sanger sequencing can still be used if NGS is unavailable.¹⁵ Furthermore, ERIC highlights the importance of accurate classification of *TP53* abnormalities – distinguishing between del(17p) and *TP53* mutations, and between monoallelic

and biallelic events. Inclusion of low-*VAF* *TP53* variants in clinical trial designs is encouraged to generate robust evidence for personalized treatment approaches.¹⁵

In previous studies, isolated low-burden *TP53* mutations have been observed in approx. 15–18% of patients with CLL.^{18,19} While biallelic *TP53* disruption, typically a *TP53* mutation accompanied by *del*(17p), is clearly associated with inferior survival, the prognostic and predictive significance of isolated high-burden *TP53* mutations remains less well defined. Inconsistent data from studies evaluating low-level *TP53* mutations in patients receiving CIT suggest that these alterations may not clearly correspond to either high- or low-risk categories, but may instead indicate an intermediate-risk profile.^{20–24} Recently, results of an ERIC-coordinated multicenter study involving our laboratory on the role of low-*VAF* variants confirmed that such variants affected outcomes in patients receiving CIT.²⁵ However, additional evidence is needed regarding the clinical impact of minor-clone *TP53* mutations in the context of novel targeted therapies.

Objectives

The aim of the study was to assess the clinical significance of *TP53* mutations in patients with relapsed/refractory CLL without *del*(17p) who were treated with ibrutinib.

Materials and methods

Study population

This retrospective analysis included a cohort of patients selected from a large observational study conducted by the Polish Adult Leukemia Study Group (PALG), entitled “Assessment of genetic and immunologic prognostic and predictive factors in relapsed and refractory chronic lymphocytic leukemia patients treated with B-cell receptor inhibitors: an observational study of the PALG”.²⁶ Centralized data collection and peripheral blood biobanking were performed in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of the Institute of Hematology and Transfusion Medicine in Warsaw, Poland (approval No. 47/2018). The inclusion criteria for the original PALG observational study, the NGS methodology, and a subset of the *TP53* sequencing results have been described previously.²⁶

For the present analysis, patients were selected based on the following criteria: 1) the presence of a *TP53* mutation detected using NGS and the absence of *del*(17p); and 2) initiation of ibrutinib treatment immediately after identification of a *TP53* mutation. *TP53* mutations were categorized as high-burden or high-*VAF* (*VAF* ≥ 10%) and low-burden or low-*VAF* (*VAF* < 10%).

Assessment of the treatment response and adverse events

Clinical data for the selected patients were retrospectively collected from PALG centers through a review of medical records. Treatment responses were assessed according to the 2018 International Workshop on Chronic Lymphocytic Leukemia (iwCLL) criteria, and the clinical response was defined as the best response achieved during the treatment period.²⁷ Adverse events (AEs) occurring during ibrutinib treatment were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, v. 5.0.²⁸

Statistical analyses

Time-to-event endpoints were progression-free survival (PFS) and overall survival (OS), measured from treatment initiation (time zero) to the corresponding event, with censoring at the last contact. Medians and 95% confidence intervals (95% CIs) were estimated using the Kaplan–Meier method, with numbers at risk reported. The proportional hazards assumption was assessed using Schoenfeld residuals. Group comparisons included: 1) the log-rank test and weighted Fleming–Harrington tests ($\rho = 0$, $\gamma = 1$ for late differences; $\rho = 1$, $\gamma = 0$ for early differences); 2) restricted mean survival time (RMST) analyses, which do not require proportional hazards, up to $\tau = 24$ months as the primary horizon, with 36 months used for a sensitivity analysis, and between-group differences estimated with 95% bootstrap CIs based on 1,000 replicates ($B = 1,000$; seed = 123); and 3) univariable Cox proportional hazards models (using the Efron method to handle ties). Analyses were performed in R v. 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria), using the survival v. 3.8-3 (<https://CRAN.R-project.org/package=survival>) and survRM2 v. 1.0-4 (<https://CRAN.R-project.org/package=survRM2>) packages, and verified using custom scripts. A $p < 0.05$ was considered statistically significant.

Results

Patients

Between November 2018 and February 2023, as part of the PALG observational study, *TP53* sequencing using NGS was performed in 468 patients with relapse/refractory CLL, all of whom tested negative for *del*(17p) (fluorescence in situ hybridization (FISH) was used). Pathogenic or likely pathogenic *TP53* mutations were identified in 76 patients (16.2%). Of these, subsequent treatment data were retrospectively available for 29 patients, of whom 25, from 11 hematology centers, received ibrutinib. The analyzed cohort included 15 men and 10 women, with a median age of 67 years (range: 58–83 years).

In the high-burden *TP53* mutation cohort, unmutated *IGHV* (um*IGHV*) was observed in 7 patients (53.8%) and mutated *IGHV* (m*IGHV*) in 2 patients (15.4%), while

Table 1. Baseline characteristics of 25 patients with RR CLL and *TP53* mutations treated with ibrutinib

Characteristics		Value	
Median age (range) [years]		67 (58–83)	
Male/female		15/10	
Binet stage at CLL diagnosis	A	12	
	B	6	
	C	6	
	not reported	1	
Number of previous therapies		3 (range: 1–7)	
Previous treatments	chemotherapy	9	
	CIT with rituximab	24	
	obinutuzumab + chlorambucil	2	
	venetoclax-based regimen	1	
	ublituximab with umbralisib (clinical trial)	1	
Autoimmune complications before ibrutinib therapy		AIHA	7
		ITP	4
ECOG performance status		0–1	18
		2–3	7
CIRS score		≤6	12
		>6	11
		not reported	2
del(17p)		0	
TP53 mutation		high-burden TP53 mutation	13 (52%)
		low-burden TP53 mutation	12 (48%)
IGHV mutational status	high-burden TP53 mutation cohort	um/IGHV	7 (53.8%)
		m/IGHV	2 (15.4%)
		borderline/nonproductive IGHV	4 (30.8%)
	low-burden TP53 mutation cohort	um/IGHV	6 (50%)
		m/IGHV	2 (16.7%)
		borderline/nonproductive IGHV	4 (33.3%)

AIHA – autoimmune hemolytic anemia; CIRS – Cumulative Illness Rating Scale; CIT – chemoimmunotherapy; CLL – chronic lymphocytic leukemia; del(17p) – deletion of the short arm of chromosome 17; ECOG – Eastern Cooperative Oncology Group; *IGHV* – immunoglobulin heavy chain variable; ITP – immune thrombocytopenia; m/*IGHV* – mutated *IGHV*; um/*IGHV* – unmutated *IGHV*.

4 patients (30.8%) had borderline or nonproductive *IGHV*. Similarly, in the low-burden cohort, um/*IGHV* was found in 6 patients (50.0%) and m/*IGHV* in 2 patients (16.7%), with 4 patients (33.3%) also having borderline or nonproductive *IGHV*. Patient characteristics are summarized in Table 1.

The *TP53* NGS analysis identified 13 patients with high-burden mutations and 12 patients with low-burden variants. All mutations were located within exons 2–11. Point mutations were the most common, although frameshift mutations were detected in 4 patients. Seven patients carried 2 distinct mutations. Detailed NGS results are provided in Table 2.

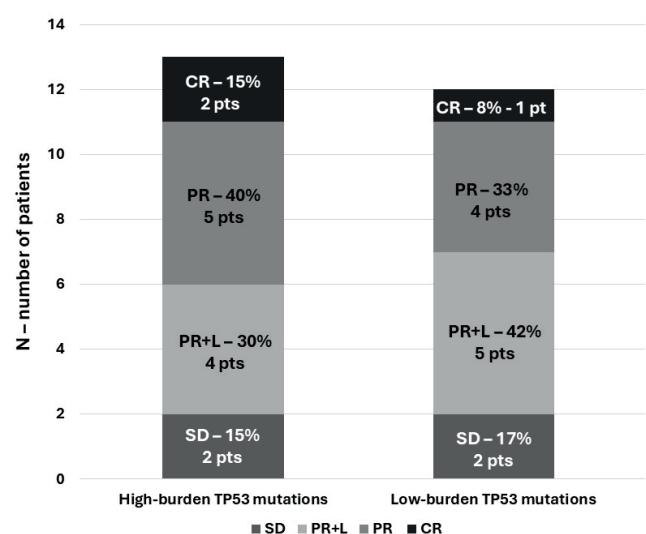
Treatment results

The median follow-up was 19 months (range: 1–40 months). The overall response rate (ORR) in the entire study cohort was 84%, and median PFS and OS were both 21 months. The 12-month PFS rate was 75.2% (95% CI: 52.7–88.0%), and the 12-month OS rate was 75.2% (95% CI: 52.7–88.0%).

Due to the small sample size, only univariable analyses were conducted to compare treatment outcomes in patients stratified by high- and low-burden *TP53* mutations. The ORR was similar in both subgroups (84.6% and 83.3% in the high- and low-burden groups, respectively) (Fig. 1). The ORR was compared between the *TP53*-high and *TP53*-low groups. Effect sizes are reported as the absolute risk difference (RD), risk ratio (RR), and odds ratio (OR) with 95% CIs. The ORR was 85% (11/13) in the *TP53*-high group vs 83% (10/12) in the *TP53*-low group. The RD was +1% (95% CI: –28% to +31%), the RR was 1.02 (95% CI: 0.72–1.45), and the OR was 1.10 (95% CI: 0.14–8.35). Fisher's exact test did not show a significant difference (group sizes: 13 and 12; OR = 1.10, 95% CI: 0.14–8.35; $p = 1.000$) (Table 3).

The median PFS in the high-burden group was 20 months, compared with 21 months in the low-burden group (hazard ratio (HR) = 0.87, 95% CI: 0.31–2.49). There were no statistically significant differences in PFS between the groups (HR = 0.74, 95% CI: 0.28–2.01; $p = 0.559$) (Fig. 2, Table 4).

Ibrutinib therapy was discontinued in 15 patients (60%). The reasons for discontinuation were AEs in 7 patients (28%), progressive disease in 7 patients (28%), and an unknown reason in 1 patient (4%). Two patients experienced Richter transformation, with diffuse large B-cell lymphoma (DLBCL) diagnosed in both cases. Adverse events leading to ibrutinib discontinuation included infections,

**Fig. 1.** Response rates to ibrutinib monotherapy in patients with CLL and high- or low-burden *TP53* mutations

CLL – chronic lymphocytic leukemia; SD – stable disease; PR+L – partial response with lymphocytosis; PR – partial response; CR – complete response.

Table 2. Next generation sequencing (NGS) results

Patient No.	High-burden <i>TP53</i> mutation (n = 13)	Patient No.	Low-burden <i>TP53</i> mutation (n = 12)
1c	Cys242AlafsTer5 (87%)	1sc	Arg175His (8%) / His193Arg (6%)
2c	Glu271Ter (81%) / Arg273His (5%)	2sc	c.560-2A>C, p.? (7%)
3c	Arg175His (73%)	3sc	Arg175Cys (7%)
4c	Met246Val (52%)	4sc	Pro278Leu (6%)
5c	Arg282GlyfsTer63 (51%) / Gly360Ala (37%)	5sc	Arg248Trp (6%)
6c	Arg248Gln (45%)	6sc	Lys164Arg (5%)
7c	Gly245Ser (30%)	7sc	Arg273Cys (5%)
8c	Thr211Ile (26%) / Gly245Arg (7%)	8sc	Arg273Ser (5%)
9c	Arg249Ser (18%) / Gln317Ter (13%)	9sc	Gly266Arg (4%) / Asp281Gly (3%)
10c	Cys176Tyr (11%)	10sc	wt/P190L (4%)
11c	Asp281Asn (11%)	11sc	Phe328SerfsTer17 (3%) / Ser241Cys (3%)
12c	Pro151Ala (11%)	12sc	Thr125= (3%)
13c	Arg175His (11%)	–	–

Table 3. Comparison of ORR between high- and low-burden *TP53* mutation groups

Group	Responders/total	Proportion	RD (95% CI)	RR (95% CI)	OR (95% CI)
<i>TP53</i> -high	11/13	0.85	+0.01 (–0.28 to +0.31)	1.02 (0.72–1.45)	1.10 (0.14–8.35)
<i>TP53</i> -low	10/12	0.83	reference	reference	reference

ORR – overall response rate; OR – odds ratio; 95% CI – 95% confidence interval; RR – risk ratio; RD – risk difference.

Table 4. Univariable Cox proportional hazards model results comparing high-burden *TP53* mutations (*TP53* = 1) with low-burden *TP53* mutations (*TP53* = 0)

Endpoint	Variable	HR (95% CI)	p-value	Concordance (C)	AIC
PFS	<i>TP53</i> (1 vs 0)	0.74 (0.28–2.01)	0.559	0.54	83.2
OS	<i>TP53</i> (1 vs 0)	0.75 (0.28–2.01)	0.562	0.54	82.9

PFS – progression-free survival; OS – overall survival; HR – hazard ratio; 95% CI – 95% confidence interval; AIC – Akaike information criterion.

mainly pneumonia (in 5 patients, 20%), including 3 cases related to COVID-19.

The median OS in the high-burden *TP53* mutation group was 22 months, compared with 21 months in the low-burden group (HR = 0.78, 95% CI: 0.28–2.19). Overall survival did not differ significantly between the groups (HR = 0.75, 95% CI: 0.28–2.01; $p = 0.562$) (Fig. 2). The most frequent causes of death among the 15 patients who died were infections (6 patients, 40%) and CLL progression (6 patients, 40%). Among infection-related deaths, COVID-19 was the confirmed cause in 4 patients (27%). The cause of death was either unknown or not reported for 3 patients (20%).

The Schoenfeld residuals test did not demonstrate a violation of the proportional hazards assumption (PFS: $p = 0.730$; OS: $p = 0.860$). However, as the Kaplan–Meier curves appeared to cross, we also report RMST estimates as summary measures that do not require proportional hazards. RMST analysis up to $\tau = 24$ months showed a restricted mean PFS of 16.7 months for *TP53* = 0 (low-burden *TP53*-mutation) vs 18.1 months for *TP53* = 1 (high-burden *TP53* mutation), with a difference of +1.4 months (95% CI: –4.8 to 10.5 months). For OS, RMST was 17.3 months vs 18.9 months, with a difference of 1.6 months (95% CI: –4.6 to 7.8 months). Sensitivity analyses at $\tau = 36$ months yielded

consistent results (PFS difference: +6.3 months; 95% CI: –8.2 to 23.3 months; OS difference: +6.5 months; 95% CI: –7.2 to 22.5 months). The RMST analysis up to 24 months did not demonstrate significant differences between *TP53* groups. The restricted mean PFS and OS were approx. 1–2 months longer in the *TP53* = 1 (high-burden *TP53* mutation) group; however, the 95% CIs included differences favoring either group as well as no difference. In the sensitivity analysis at 36 months, differences increased to about 6 months yet remained statistically nonsignificant, with wide CIs. Additionally, Fleming–Harrington weighted tests were consistent with the log-rank test, showing no significant differences between groups (PFS: FH(0.1) $p = 0.392$; FH(1.0) $p = 0.701$; OS: FH(0.1) $p = 0.399$; FH(1.0) $p = 0.701$). These results support the finding that no statistically significant differences between the survival curves were detected.

The observed toxicity profile of ibrutinib was consistent with previous reports. The most frequent AEs observed during ibrutinib therapy included infections (mainly pneumonia and COVID-19) and diarrhea. Overall, 12 patients (48%) experienced at least 1 grade 3 or 4 AE. Infections of any grade were reported in 21 patients (84%), including 4 cases of COVID-19 (16%). Diarrhea was observed in 5 patients (20%), all with grade 2 events. Rash was

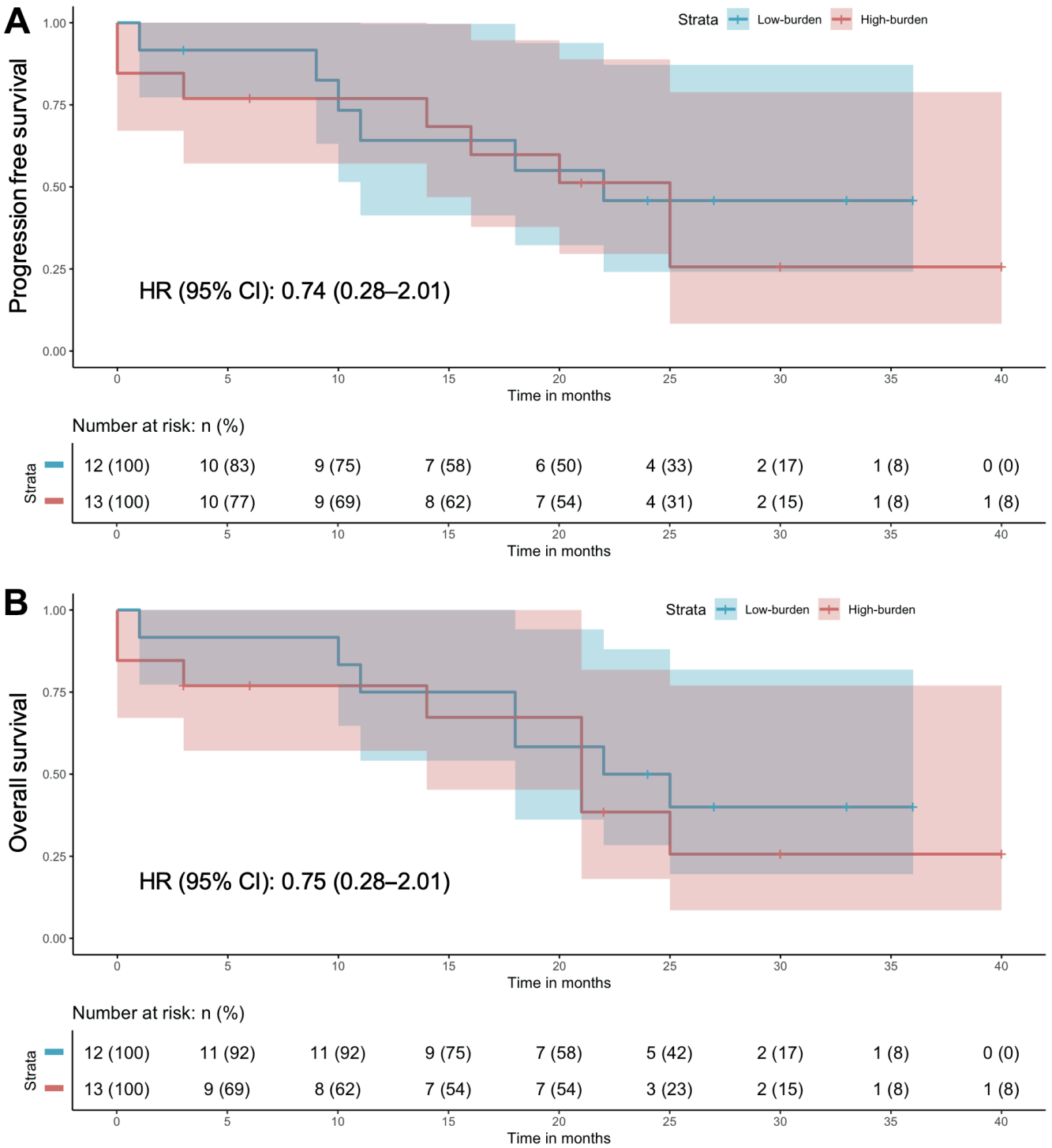


Fig. 2. Kaplan–Meier curves showing PFS (A) and OS (B) in patients with CLL and high-burden (variant allele frequency (VAF) $\geq 10\%$) or low-burden (VAF $< 10\%$) *TP53* mutations

CLL – chronic lymphocytic leukemia; OS – overall survival; PFS – progression-free survival.

reported in 2 patients (8%), and bleeding-related AEs occurred in 2 patients (8%), one of whom experienced severe epistaxis (grade 3). Atrial fibrillation occurred in 2 patients (8%), with both events classified as grade 2. Most AEs resolved without the need for treatment interruption. However, 7 patients (28%) required ibrutinib dose reductions, primarily due to recurrent infections and thrombocytopenia.

Discussion

Given the uncertain significance of isolated minor-clone *TP53* mutations in the context of targeted therapies in CLL, our study aimed to compare the impact of isolated low-burden and high-burden *TP53* mutations on the efficacy of ibrutinib in patients with relapse/refractory CLL treated in routine clinical practice. We found that in heavily pretreated

patients with relapse/refractory CLL and an isolated *TP53* mutation, PFS was unsatisfactory despite a relatively high response rate to ibrutinib. Our results did not identify statistically significant associations between *TP53* mutant clone burden and the likelihood of response to ibrutinib or survival in this patient population.

TP53 abnormalities, including both deletions (del(17p)) and mutations at the *TP53* locus, are associated with an adverse prognosis and predict chemoresistance in CLL.²⁹ Ibrutinib has demonstrated the ability to induce apoptosis and disrupt BCR signaling independently of p53 function.³⁰ This suggests that its antitumor effect may not rely on intact p53 pathways. Preclinical studies support the notion that ibrutinib can bypass p53-dependent apoptotic signaling, which could explain its activity in CLL with *TP53* mutations or deletions.^{30,31} Nevertheless, the precise mechanisms remain under investigation, and further research is warranted to determine whether these agents modulate p53 function or act through entirely alternative pathways.

The most recent guidelines from the *TP53* network of ERIC recommend precise classification of *TP53* aberrations (del(17p) vs *TP53* mutation, monoallelic vs biallelic aberrations), although a specific VAF cutoff for reporting *TP53* mutations is no longer recommended. Instead, the guidelines emphasize the need for thorough method validation or verification, especially for detecting low-VAF *TP53* mutations.¹⁵ In many published studies examining new therapies in CLL, del(17p) and *TP53* mutations were not distinguished,^{32–34} while in others, only del(17p) was considered.³⁵ In the context of targeted treatments, patients harboring both *TP53* mutations and del(17p) tend to have less favorable prognoses, indicating an independent negative prognostic impact.³⁶

In contrast, the clinical significance of single-hit *TP53* aberrations (isolated del(17p) or *TP53* mutation only) remains less clear.^{36–39} Studies have shown that treatment-naïve (TN) patients with CLL and del(17p) or high-burden *TP53* mutations who receive ibrutinib have shorter OS than patients without these aberrations.^{37,40} However, recent analyses have indicated that single-hit *TP53* aberrations (either a single *TP53* mutation or del(17p) alone) in patients with CLL treated with ibrutinib are associated with better outcomes than multi-hit *TP53* aberrations.^{36,41} Furthermore, evidence regarding the significance of isolated low-burden *TP53* mutations in the context of targeted therapies remains limited, and the results are inconsistent.^{36,40,42}

Our results are consistent with the findings of Bomben et al.,³⁷ who analyzed a larger cohort of patients with TN or relapse/refractory CLL, including 60 patients with isolated *TP53* mutations (28 with low-burden mutations) treated with ibrutinib. As in our study, no differences in OS or PFS were observed between the low- and high-VAF *TP53* mutation groups. Importantly, patients with *TP53* mutations, regardless of whether VAF was <10% or ≥10%, had shorter OS than those with *TP53* wild-type disease. Cherng et al.⁴⁰ presented a retrospective analysis of 130 patients

with CLL and baseline *TP53* alterations who received first-line Bruton tyrosine kinase inhibitor (BTKi)-based therapy. This analysis showed favorable 4-year PFS and OS rates of 72.9% and 83.6%, respectively, with no significant difference in PFS among patients with both del(17p) and *TP53* mutations, del(17p) only, or *TP53* mutation only. The clone size (VAF) of *TP53* mutations was not associated with PFS (HR = 1.0, 95% CI: 0.34–2.88). However, this study did not report a comparison of isolated low-burden and high-burden *TP53* mutations.⁴⁰ In another study, Brieghel et al.³⁶ analyzed *TP53* alterations in 51 patients with TN or relapse/refractory CLL treated with ibrutinib. They found that a VAF cutoff of 1% was an appropriate threshold for *TP53* mutation “positivity,” maintaining the predictive value of *TP53* mutations. Patients with a single *TP53* aberration (either del(17p) or a *TP53* mutation) had superior PFS and OS to those with multiple *TP53* alterations. However, a limitation of the study was the lack of stratification by *TP53* clone size, along with the inclusion of both newly diagnosed and previously treated individuals.³⁶

Importantly, our results can also be contrasted with the findings of Pula et al.,⁴³ who reported the outcomes of a large, real-world cohort of patients with relapsed/refractory CLL treated with ibrutinib from the PALG. In that multicenter observational study, which included 171 patients, the median PFS and OS in the entire cohort were not reached, and the estimated 4-year PFS and OS rates were 60.9% and 65.4%, respectively – substantially more favorable than in our population, in which median PFS and OS were both 21 months.

In the subgroup of 42 patients with del(17p), Pula et al. reported a median PFS of 38.9 months and a median OS of 52.9 months – still more favorable than those observed in our group (PFS: 21 months; OS: 21 months). Several factors may have contributed to these differences, including variations in baseline characteristics, such as greater prior treatment exposure and a higher comorbidity burden in our cohort. Importantly, the study by Pula et al. was conducted before the COVID-19 pandemic, whereas our analysis includes patients treated during the pandemic period, which may have adversely affected clinical outcomes. In our cohort, COVID-19-related complications were a leading cause of death and a major reason for treatment discontinuation. This factor may have contributed substantially to the shorter survival observed, particularly in a patient population already characterized by immunosuppression and frailty.

In addition to *TP53* mutations, other factors may have contributed to the unfavorable prognosis in our study. These include extensive prior treatment exposure (a median of 3 previous lines of therapy), poor performance status (28% of patients had an ECOG performance status score >1), and a substantial burden of comorbidities (a median Cumulative Illness Rating Scale (CIRS) score of 6). Furthermore, treatment occurred during the COVID-19 pandemic, which may have adversely affected outcomes. Infections, potentially exacerbated by both disease-related

and treatment-related immunosuppression, were a joint leading cause of death in this cohort (6 of 15 deaths, 40%), with COVID-19-related pneumonia confirmed in 4 patients (27% of deaths). Importantly, pneumonia was also the most frequent AE leading to permanent discontinuation of ibrutinib (5 patients, 20%), including 3 COVID-19-related cases. Ibrutinib itself may also contribute to increased susceptibility to infections through its immunomodulatory and immunosuppressive effects. Several studies have reported an increased incidence of both opportunistic infections and severe bacterial, fungal, or viral infections – including respiratory tract infections – during ibrutinib therapy, particularly in previously treated patients or those with comorbidities.^{44,45}

Limitations of the study

This study highlights an important aspect of the prognostic relevance of isolated *TP53* mutations in patients with CLL treated with ibrutinib. Despite its strengths, several limitations must be acknowledged. Most notably, the relatively small sample size reduces statistical power, widens the CIs for HR estimates, and precludes robust conclusions regarding subgroup differences. The absence of multivariable adjustment further limits the ability to distinguish the association between *TP53* mutation burden and survival outcomes from the effects of potential confounders, such as *IGHV* mutation status, comorbidities, and patient performance status. Additionally, the retrospective design introduces the potential for bias, and the influence of treatment timing during the COVID-19 pandemic cannot be ruled out. Despite these limitations, our study adds to the growing body of evidence suggesting that *TP53* clone size may not be a decisive factor in predicting outcomes during ibrutinib treatment, at least in relapse/refractory CLL.

Conclusions

The results of our analysis did not reveal statistically significant differences in ibrutinib outcomes between patients with isolated low- and high-burden *TP53* mutations. However, given the retrospective nature of the study and the limited sample size, these observations should be interpreted with caution. Further prospective studies involving larger patient cohorts are warranted to assess the association between mutation burden and treatment outcomes and to clarify the prognostic and predictive significance of *TP53* mutation burden in the context of BTKi therapy in CLL.

Data Availability Statement

The datasets supporting the findings of the current study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.18014723>.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Noninvasive evaluation of liver fibrosis in newly diagnosed pediatric Crohn's disease: Elastography and fibrotic biomarkers at diagnosis

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Conflict of interest

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Abstract

Background. Crohn's disease (CD) is increasingly diagnosed in children, a population in which hepatic dysfunction and coexisting liver conditions are not uncommon. These factors may contribute to the development of hepatic fibrosis.

Objectives. This study aimed to evaluate liver involvement in pediatric CD at diagnosis and to explore the utility of noninvasive markers, including fibroblast growth factor 19 (FGF-19) and connective tissue growth factor (CTGF), in detecting early fibrotic changes.

Materials and methods. Fifty children with newly diagnosed CD and 23 healthy controls without gastrointestinal disorders were enrolled. All participants underwent liver function testing, noninvasive fibrosis scoring, serum FGF-19 and CTGF measurement, and liver elastography.

Results. No patient in either group demonstrated significant hepatic fibrosis (>F2 on the METAVIR scale). No statistically significant differences were found in liver elastography results between the study group and the control group (3.98 kPa vs 3.92 kPa, $p = 0.430$). A positive correlation was found between liver stiffness values and fecal calprotectin levels, suggesting a link to intestinal inflammation. Compared to controls, CD patients had significantly lower serum FGF-19 (87.8 pg/mL vs 123.7 pg/mL, $p = 0.028$) and higher CTGF concentrations (710.8 pg/mL vs 252.2 pg/mL, $p < 0.001$).

Conclusions. Elevated liver stiffness was uncommon at the time of CD diagnosis in pediatric patients; no patients had findings suggestive of advanced fibrosis ($\geq F3$), and only 1 patient had liver stiffness corresponding to F2 fibrosis. Altered levels of FGF-19 and CTGF may reflect intestinal inflammation and a potential fibrotic response within the gut rather than the liver. Elevated elastography values appear to be influenced by intestinal disease activity, underscoring the importance of distinguishing hepatic from intestinal sources of biomarker changes in this population.

Key words: children, elastography, liver fibrosis, FGF-19, CTGF

Highlights

- Advanced liver fibrosis was not detected at the time of Crohn's disease (CD) diagnosis in pediatric patients using liver elastography and noninvasive fibrosis assessment.
- Children with newly diagnosed CD had significantly lower serum fibroblast growth factor 19 (FGF-19) and higher connective tissue growth factor (CTGF) levels than healthy controls, suggesting altered fibrotic signaling.
- Liver stiffness measured using elastography positively correlated with fecal calprotectin, indicating that intestinal inflammation may influence liver fibrosis assessment.
- FGF-19 and CTGF may serve as noninvasive biomarkers of intestinal inflammation and early fibrotic responses in pediatric CD rather than hepatic fibrosis.

Background

In recent years, a rising incidence of Crohn's disease (CD) among children has been observed.^{1,2} It is an autoimmune disorder that can affect any part of the gastrointestinal tract, most commonly involving the terminal ileum. As CD is a chronic, autoimmune condition, it requires special attention, particularly in terms of identifying potential complications and comorbidities. Hepatic dysfunction is quite common in pediatric patients with CD.³ According to available data, elevated transaminase activity is observed in up to 30% of children with CD across various disease stages. This elevation may be related to the therapeutic regimen or coexisting disorders or may represent an extraintestinal manifestation of the disease.^{4–6} Special consideration should be given to some other autoimmune, potentially coexisting diseases, especially primary sclerosing cholangitis (PSC), but also autoimmune hepatitis (AIH) and AIH-PSC overlap syndrome, which may coexist with CD and require appropriate clinical management. According to the existing guidelines, patients diagnosed with CD should be monitored for the presence of these associated disorders, especially PSC.⁷

To date, liver biopsy remains the gold standard for diagnosing liver diseases, including those leading to fibrosis, across all age groups. Although the procedure is brief, it requires general anesthesia in pediatric patients and carries risks of complications such as bleeding or bile duct injury. Despite these limitations, it is essential for the assessment of histopathological changes,^{8,9} including the presence and severity of fibrosis. Nonetheless, alternative, less invasive methods for evaluating liver fibrosis are increasingly being adopted in clinical practice.

Due to the need for noninvasive and minimally invasive methods to assess the presence and severity of liver fibrosis in children, ongoing efforts are being made to identify optimal alternatives to invasive procedures. Liver fibrosis indices such as fibrosis-4 (FIB-4) and AST-to-platelet ratio index (APRI), commonly used in adult populations, appear to have potential applicability in pediatric patients as well.^{10–12}

A simple method for assessing fibrosis in patients with CD may also involve measuring fibrosis-related biomarkers, such as fibroblast growth factor 19 (FGF-19) and connective tissue growth factor (CTGF), which may exert profibrotic effects on hepatic cells.^{13,14} FGF-19 is a protein produced in the ileum that plays a key role in regulating bile acid synthesis,¹⁵ and its serum level may be associated with the degree of liver disease progression.¹⁶ CTGF is involved in fibrosis development in various organs, including the lungs, kidneys, and liver. It also plays a role in tumor progression, angiogenesis, and cartilage formation.¹⁴

Another noninvasive method for assessing liver fibrosis is ultrasound elastography, whose results correlate well with tissue stiffness as determined by biopsy.¹⁷ This technique has already been used in adult patients with CD^{18–20} and has also been applied as a diagnostic tool in various pediatric conditions.^{21–23} Although ultrasound elastography is a simple, accessible, and noninvasive method, its role in diagnosing liver diseases in pediatric patients with CD has not yet been established. It should also be noted that this method has certain limitations – successful measurement requires patient cooperation, which may be challenging in very young children. Although CD predominantly affects individuals in young adulthood, pediatric patients are generally able to cooperate, making this diagnostic technique a simple and practical option in this population.

Objectives

The primary aim of the study was to assess the presence and severity of liver fibrosis in children with newly diagnosed CD using noninvasive methods such as liver fibrosis indices (APRI and FIB-4), fibrosis biomarkers (FGF-19 and CTGF), and liver elastography. The secondary aim of our study was to assess the presence and severity of hepatic fibrosis in relation to the location of gastrointestinal inflammatory lesions at the time of diagnosis.

Materials and methods

Study participants

A single-center prospective study was conducted, including 50 pediatric patients with newly diagnosed CD and no previously identified liver disease, as well as a control group of 23 patients without any previously diagnosed chronic gastrointestinal disorders. The control patients were suspected of having gastrointestinal diseases, which were subsequently excluded based on diagnostic evaluation. They showed no signs of intestinal or hepatic inflammation, as determined in laboratory and imaging studies. All participants were hospitalized at the Department of Pediatrics and Gastroenterology, University Children's Hospital (Cracow, Poland) between May 2022 and November 2024. The mean age of patients was 12.8 years in the study group and 13.2 years in the control group.

The study was conducted in accordance with the Declaration of Helsinki; informed consent was obtained from the parents of all participants and from patients aged 13 years and older. The study protocol was approved by the Bioethics Committee at Jagiellonian University in Cracow, Poland (approval No. KBET 1072.6120.101.2021).

Data collection and laboratory assessment

Following the diagnosis of CD, patients in the study group underwent baseline biochemical testing in a fasting state, including liver fibrosis assessment using FIB-4 (calculated from alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelet count (PLT), and age) and APRI (based on AST, the upper limit of normal for AST, and PLT). The same tests were conducted on healthy individuals in the control group using an identical protocol. CTGF measurements were performed using the Human Connective Tissue Growth Factor (CTGF) Enzyme-Linked Immunosorbent Assay (ELISA) Kit (DLdevelop, Wuxi, China), and FGF-19 measurements were performed using the Human FGF-19 Quantikine ELISA (R&D Systems, Minneapolis, USA). Blood samples for laboratory determinations were collected in the morning from fasting participants in both groups. The measurements were performed in serum samples.

Elastography technique

Liver elastography was performed with the point shear-wave method on the Arietta V70 (Hitachi Aloka, Slough, UK) using a convex C251 probe (1.8–5.0 MHz). All examinations in both patient groups were conducted by a single investigator after the patients had fasted for at least 3 h. Measurements were obtained via an intercostal approach. For each patient, 10 measurements were taken, and the median value was expressed in kilopascals (kPa)

and interpreted according to the METAVIR scoring system: F0 < 6.5 kPa (no fibrosis), F1 < 9.1 kPa (mild fibrosis), F2 < 10.8 kPa (moderate fibrosis), F3 < 13.3 kPa (advanced fibrosis), and F4 ≥ 13.3 kPa (cirrhosis).²⁴ Measurements were considered valid when the interquartile range-to-median ratio (IQR/median) was <0.3. All procedures were performed in accordance with the EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017.²⁵

Statistical analyses

All analyses were performed on original, non-transformed data. Quantitative variables were analyzed using descriptive statistics, including mean, standard deviation (SD), median, quartiles, and minimum and maximum values. Qualitative variables were analyzed by calculating absolute and relative frequencies for all possible values. Comparisons of qualitative variables between groups were performed using Pearson's χ^2 test for independence (with Yates's correction for 2×2 tables) or Fisher's exact or Fisher–Freeman–Halton test when the assumptions of the χ^2 test regarding expected frequencies were not met. Comparisons of quantitative variables between 2 groups were conducted using the Mann–Whitney U test. For comparisons involving 3 or more groups, the Kruskal–Wallis test was applied, followed by Dunn's post hoc test when differences were statistically significant. Correlations between quantitative variables were assessed using Spearman's rank correlation. Prior to correlation analysis, the monotonicity assumption was evaluated through visual inspection of scatter plots. No substantive non-monotonic patterns (e.g., U-shaped or inverted U-shaped relationships) were observed; therefore, nonparametric correlations were considered appropriate. Diagnostic plots used to assess distributional characteristics and monotonicity are provided in Supplementary data (Supplementary Fig. 1–40). A significance level of 0.05 was adopted for all analyses; thus, p-values below 0.05 were considered statistically significant. Each statistical comparison corresponded to a separate, predefined hypothesis rather than to a single overarching test; therefore, no multiplicity correction was applied. This analytical framework aligns with interpreting each association as an independent inferential question. Nevertheless, the potential inflation of the type I error rate inherent in multiple independent tests is acknowledged, and borderline p-values should be interpreted with caution. Missing data were handled using an available-case (pairwise deletion) approach. No imputation procedures were applied. For each statistical test or correlation, only observations with complete data for the variables involved were included; therefore, sample sizes vary across analyses. Statistical analyses were performed using R v. 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

Results

Patients' characteristics

The study included 50 patients (22 girls and 28 boys) in the study group and 23 patients (13 girls and 10 boys) in the control group. No statistically significant differences were observed between the groups in terms of age, sex, or body mass index (BMI; detailed data are presented in Table 1).

In the study group, significantly lower levels of hemoglobin and albumin were observed, along with significantly increased inflammatory markers (PLT, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), calprotectin) and gamma-glutamyl transpeptidase (GGT), with no differences in AST and ALT. Additionally, in this group of patients, 4 had elevated GGT levels, and only 1 patient had increased transaminase values ($>2\times$ the upper limit of normal) at the time of diagnosis. A comparison of laboratory test results in the study group is presented in Table 2.

Disease characteristics in patients with newly diagnosed CD were assessed using the Paris classification,²⁶ while disease severity was evaluated using the Pediatric Crohn's Disease Activity Index (PCDAI).²⁷ The results are presented in Table 3.

Liver fibrosis assessment

In the studied cohort, elastography values corresponding to METAVIR stage F2 were identified in only 1 patient. This patient also presented with elevated GGT levels; however, subsequent diagnostic work-up excluded liver disease. All remaining patients demonstrated liver elastography values not exceeding F2. In those with elastography readings above 6.5 kPa, mildly elevated ALT and AST levels were noted – remaining below twice the upper limit of normal – alongside increased GGT concentrations. In the control group, abnormal elastography values (>6.5 kPa) were noted in 2 participants, whose liver enzyme levels remained within reference ranges. Elastography values did not differ significantly between the groups. Liver fibrosis indices were significantly higher in the control group compared to the study group. However, none of the patients had values indicative of liver fibrosis. With respect to fibrosis-related proteins, FGF-19 levels were significantly reduced in the study group, whereas CTGF levels were significantly increased. Liver fibrosis parameters are detailed in Table 4.

Table 1. Demographic and anthropometric characteristics of the study and control groups, including age, sex distribution, and body mass index (BMI)

Parameter		Study group (n = 50)	Control group (n = 23)	p-value
Age [years]	median (Q1–Q3)	14 (9.75–15)	14.5 (10.75–16.25)	M-W: n = (50, 23); p = 0.536
Sex	females	22 (44.00%)	13 (56.52%)	χ^2 test: df = 1; p = 0.458
	males	28 (56.00%)	10 (43.48%)	
BMI [kg/m ²]	median (Q1–Q3)	17.1 (15.3–19.2)	19.2 (16–21.4)	M-W: n = (47, 21); p = 0.064

M-W – Mann–Whitney U test; df – degrees of freedom; IQR – interquartile range.

Table 2. Basic laboratory parameters in both groups. All results are presented as median (Q1–Q3)

Parameter	Study group (n = 50)	Control group (n = 23)	p-value
WBC [$10^3/\mu\text{L}$]	7.85 (6.57–10)	7.17 (5.82–8.37)	M-W: n = (50, 23); p = 0.146
RBC [$10^6/\mu\text{L}$]	4.78 (4.31–4.99)	4.62 (4.44–4.79)	M-W: n = (50, 23); p = 0.245
Hgb [g/dL]	11.85 (10.2–13.4)	13.3 (12.45–14.3)	M-W: n = (50, 23); p = 0.001
Hct [%]	35.85 (32.32–39.2)	38.1 (36.2–40.35)	M-W: n = (50, 23); p = 0.021
MCV [fL]	76.9 (72.8–80.75)	82.7 (78.6–87.05)	M-W: n = (50, 23); p < 0.001
PLT [$10^3/\mu\text{L}$]	409.5 (336–469.75)	308 (280–354)	M-W: n = (50, 23); p < 0.001
CRP [mg/L]	5.01 (5–19.5)	5 (5–5)	M-W: n = (49, 23); p = 0.001
ESR [mm/h]	20 (10–32.5)	6 (3–8)	M-W: n = (47, 15); p = 0.001
ALT [U/L]	16 (13–26.75)	18 (15–21.5)	M-W: n = (50, 23); p = 0.712
AST [U/L]	26.3 (21.5–32.92)	26.4 (23.05–31.45)	M-W: n = (50, 23); p = 0.910
GGT [U/L]	15 (12–22.25)	12 (11–14.75)	M-W: n = (50, 22); p = 0.017
Albumin [g/L]	41.95 (36.75–45.1)	46 (43.15–47.75)	M-W: n = (50, 23); p = 0.002
Vitamin D [ng/mL]	28.8 (21.3–32.4)	22.6 (18.9–26)	M-W: n = (33, 17); p = 0.049
Calprotectin [$\mu\text{g/g}$]	2130 (948–3630)	39 (20–84)	M-W: n = (49, 12); p < 0.001

WBC – white blood cells; RBC – red blood cells; Hgb – hemoglobin; Hct – hematocrit; MCV – mean corpuscular volume; PLT – platelets; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; IQR – interquartile range; M-W – Mann–Whitney U test. Values in bold are statistically significant.

Table 3. Characteristics of patients diagnosed with CD according to disease location based on the Paris classification and disease severity assessed using the PCDAI

Characteristic		Category	Number of patients with CD
Paris classification	age [years]	<10 (A1a)	13 (26%)
		10–17 (A1b)	37 (74%)
		>17 (A2)	0 (0%)
	location	distal 1/3 ileal and/or limited cecal disease (L1)	1 (2%)
		colonic (L2)	18 (36%)
		ileocolonic (L3)	31 (62%)
		upper disease proximal to ligament of Treitz (L4a)	41 (82%)
		upper disease distal to ligament of Treitz and proximal to 1/3 ileum (L4b)	2 (4%)
	behavior	non-stricturing, non-penetrating (B1)	43 (86%)
		stricturing (B2)	4 (8%)
		penetrating (B3)	1 (2%)
		stricturing and penetrating (B2B3)	2 (4%)
		perianal disease (P)	4 (8%)
	growth	no growth delay (G0)	46 (92%)
		growth delay (G1)	4 (8%)
PCDAI [points]		remission (<10)	11 (22%)
		mild (10–27.5)	24 (48%)
		moderate (30–37.5)	9 (18%)
		severe (>= 40)	6 (12%)

CD – Crohn's disease; PCDAI – Pediatric Crohn's Disease Activity Index.

Table 4. Summary of parameters used for the noninvasive assessment of liver fibrosis

Parameter		Study group	Control group	p-value
Elastography value [kPa], median (Q1–Q3)		3.98 (3.56–4.9)	3.92 (3.38–4.73)	M-W: n = (50, 23); p = 0.430
METAVIR, n (%)	F0	47 (94%)	21 (91.3%)	Fisher's exact test: p = 0.718
	F1	2 (4%)	2 (8.7%)	
	F2	1 (2%)	0	
	F3	0	0	
	F4	0	0	
FIB-4 [points], median (Q1–Q3)		0.205 (0.120–0.273)	0.260 (0.220–0.360)	M-W: n = (50, 23); p = 0.012
APRI [points], median (Q1–Q3)		0.164 (0.122–0.213)	0.199 (0.158–0.246)	M-W: n = (50, 23); p = 0.023
FGF-19 [pg/mL], median (Q1–Q3)		87.8 (55.4–134.9)	123.7 (79.9–232.2)	M-W: n = (49, 21); p = 0.028
CTGF [pg/mL], median (Q1–Q3)		710.8 (389.3–929.7)	252.2 (94.6–344.6)	M-W: n = (49, 21); p < 0.001

M-W – Mann–Whitney U test; FIB-4 – fibrosis-4; APRI – AST to Platelet Ratio Index; CTGF – connective tissue growth factor; FGF-19 – fibroblast growth factor-19.

Correlations between elastography, fibrotic markers, and biochemical parameters

Elastography

The median liver elastography values and IQRs Q1–Q3) were 3.98 kPa (3.56–4.90 kPa) in the study group and 3.92 kPa (3.38–4.73 kPa) in the control group, and the difference between groups was not statistically significant ($p = 0.430$). In the study group, a statistically significant positive correlation was observed between liver elastography values and fecal calprotectin levels ($\rho = 0.310$,

95% confidence interval (95% CI): 0.03 to 0.54, $n = 49$, $p = 0.036$). No significant correlations were found between elastography and other inflammatory markers, including CRP ($n = 49$), ESR ($n = 47$), or PLT ($n = 50$). In the control group, a statistically significant negative correlation was noted between elastography and ESR ($\rho = -0.692$, 95% CI: -0.89 to -0.28 , $n = 15$, $p = 0.004$), while no significant associations were found with CRP ($n = 23$), PLT ($n = 23$), or calprotectin ($n = 12$). Across both groups, no significant correlations were observed between elastography values and liver enzyme activity (ALT, AST, and GGT). Additionally, no statistically significant differences in elastography or METAVIR scores were found between the study and control groups. No significant correlations

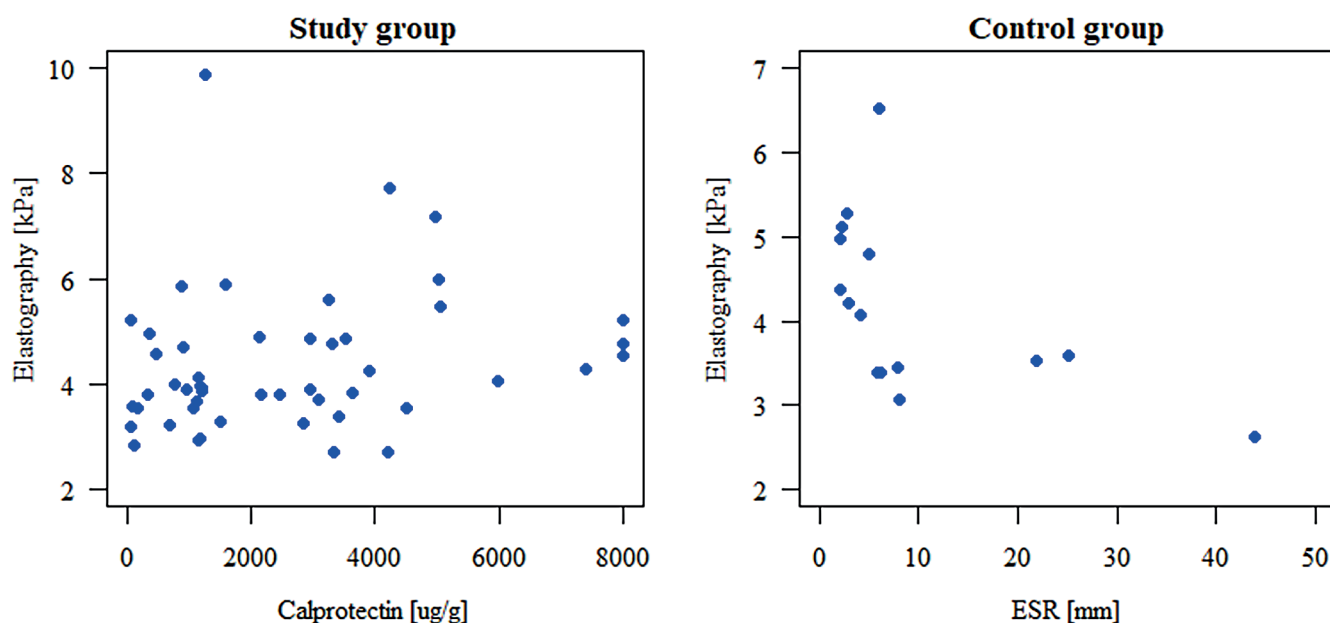


Fig. 1. Correlations between liver stiffness and laboratory parameters: calprotectin in the study group and erythrocyte sedimentation rate (ESR) in the control group

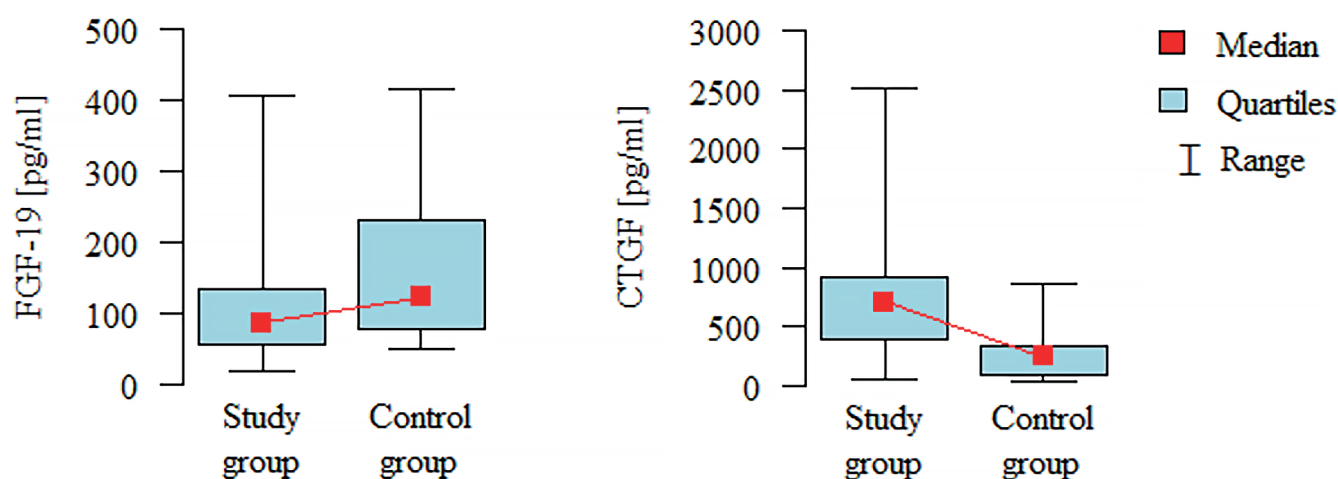


Fig. 2. Differences in fibroblast growth factor-19 (FGF-19) and connective tissue growth factor (CTGF) levels between the study and control groups

were observed between elastography and liver fibrosis indices (FIB-4 and APRI) in either group. Elastography values were also not associated with disease severity as assessed using PCDAI. Correlations with liver elastography are illustrated in Fig. 1. The remaining correlation plots have been included in the Supplementary data (Supplementary Fig. 1–6 and 21–26).

FGF-19 and CTGF

Statistically significant differences in fibrosis-related protein levels were observed between the study and control groups. Patients in the study group had significantly lower serum levels of FGF-19 compared to controls (Mann–Whitney (M-W) test: $n = (49, 21)$; $p = 0.028$), while CTGF levels were significantly higher in the study group (M-W test: $n = (49, 21)$; $p < 0.001$). No significant correlations

were found between FGF-19 or CTGF levels and inflammatory markers (CRP, ESR, PLT, and calprotectin) or liver enzyme activity (ALT, AST, and GGT) in either group. Furthermore, no associations were observed between protein levels and disease severity as measured using PCDAI. Differences in the concentrations of FGF-19 and CTGF proteins between the study and control groups are shown in Fig. 2. The remaining correlation plots are included in the Supplementary data (Supplementary Fig. 7–20 and 27–40).

Subgroup analysis based on Paris classification

When patients were stratified according to the Paris classification, significant differences in FIB-4 and CTGF values were observed across age subgroups. Higher FIB-4 values were found in patients classified as A1b compared

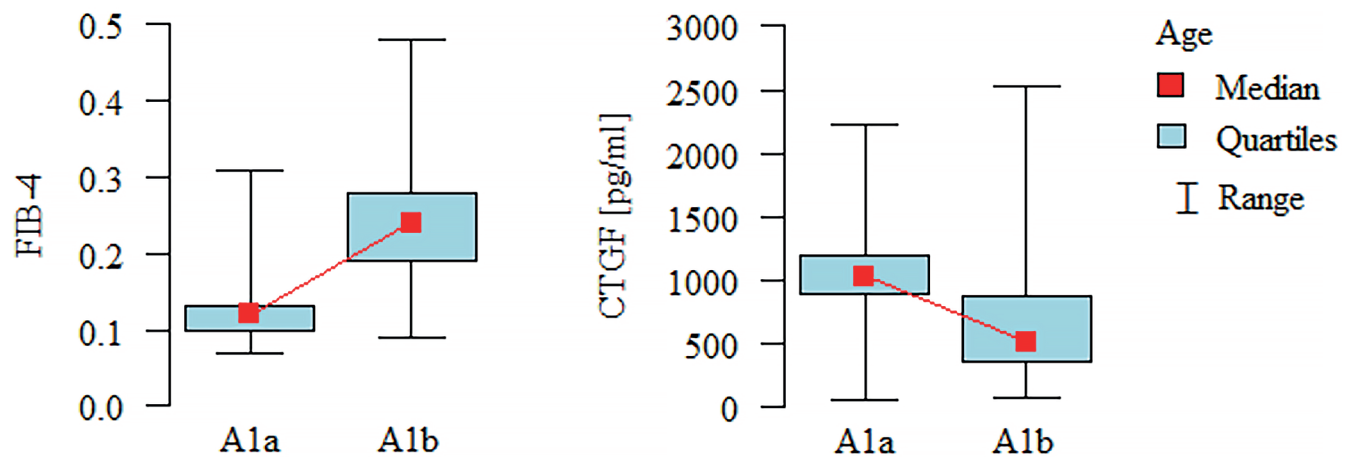


Fig. 3. Differences in fibrosis-4 (FIB-4) and connective tissue growth factor (CTGF) values between age groups according to the Paris classification: A1a (<10 years) and A1b (10–17 years)

to those classified as A1a (M-W test: $n = (13, 37)$; $p = 0.001$), while CTGF levels were significantly higher in the A1a subgroup (M-W test: $n = (13, 36)$; $p = 0.002$). No significant differences were noted for elastography, FGF-19, or APRI values across age categories. No significant associations were found between disease location and any other parameters assessed using the Paris classification. The difference in APRI values between patients with inflammatory disease behavior (B1) and those in other groups was of borderline statistical significance (M-W test: $n = (43, 7)$; $p = 0.049$), whereas no significant differences were observed for FIB-4, FGF-19, CTGF, or elastography. No significant differences in the analyzed markers were observed with respect to growth impairment status. Differences in FIB-4 and CTGF values across individual age groups are presented in Fig. 3.

Discussion

Our study aimed to evaluate whether pediatric patients with newly diagnosed CD exhibit signs of liver fibrosis at the time of diagnosis, using noninvasive methods including liver ultrasound elastography and serum measurements of FGF-19 and CTGF. To the best of our knowledge, the usefulness of ultrasound liver elastography and the assessment of these fibrosis-related proteins have not previously been investigated in this patient population.

To date, liver biopsy remains the gold standard for diagnosing hepatic fibrosis, with histopathological evaluation of the biopsy specimen being the most accurate method for assessing fibrotic changes in the liver.²⁸ Although biopsy is often unavoidable in patients with suspected fibrosis, there is a growing need for less invasive techniques to evaluate the presence and severity of fibrotic alterations.^{29,30} One such method is ultrasound elastography, which has demonstrated good concordance with biopsy results, mainly in the adult population,^{31–33} but also in children.³⁴ Despite its limitations – such as the need for patient

cooperation, specialized equipment, and operator expertise – elastography is increasingly used in the diagnosis and monitoring of liver diseases.

In pediatric patients with CD, hepatic dysfunction is frequently observed at various stages of the disease and may lead to chronic liver disease.^{35,36} These findings suggest that significant liver fibrosis is uncommon at disease onset. However, CD diagnosis in pediatric patients is typically established relatively early after symptom onset; longitudinal follow-up is warranted to assess the potential development of fibrotic changes over time.

Of particular interest is the observed correlation between liver stiffness and fecal calprotectin levels, which may indicate that increased hepatic stiffness is associated with intestinal inflammation. These findings may suggest that inflammatory involvement of the intestine is associated with alterations in hepatic tissue characteristics even in the absence of fibrosis. However, this relationship requires further studies to confirm its existence and to elucidate the underlying mechanisms. Elevated fecal calprotectin levels have previously been reported in patients with non-alcoholic fatty liver disease (NAFLD, currently referred to as metabolic dysfunction-associated fatty liver disease (MASLD)) and obesity,³⁷ which may indicate a link between inflammation of the liver and intestines.

To investigate the presence of liver fibrosis in our patient cohort, we also measured 2 proteins – FGF-19 and CTGF – both implicated in fibrotic processes, including hepatic fibrosis.^{13,14,38} In the study group, FGF-19 levels were significantly lower than in controls, whereas CTGF levels were significantly higher. No correlation was found between the concentrations of these proteins and liver elastography values. The lower FGF-19 and higher CTGF levels detected in the study group may indicate that fibrosis is present not in the liver but elsewhere, e.g., in the intestines, where the inflammatory process is ongoing. However, further studies assessing the presence of intestinal fibrosis at the time of diagnosis would be required to confirm this possibility. Previous studies have shown

reduced FGF-19 levels in patients with intestinal failure and liver diseases, often associated with ongoing inflammation or intestinal resection. Such a decrease in FGF-19 may contribute to the development of hepatic disorders, suggesting that CD patients with low FGF-19 levels should be monitored for potential liver involvement over time.^{39,40} Reduced levels of FGF-19 have also been observed in adult patients with inflammatory bowel disease, with its concentration inversely correlated with the severity of inflammation,⁴¹ and this observation may be related to the expression of the farnesoid X receptor, which is predominantly located in the ileum.^{42,43} Similarly, elevated CTGF expression has been reported in inflamed intestinal segments of patients with inflammatory bowel diseases, including CD, supporting its role in intestinal fibrosis. Accordingly, increased CTGF concentrations in newly diagnosed CD may reflect ongoing inflammatory or fibrotic processes within the intestinal wall driven by inflammation.^{44,45}

FIB-4 and APRI scores were also assessed in all patients. None of the evaluated individuals had values indicative of liver fibrosis. Interestingly, both indices were significantly higher in the control group compared to the study group, which was most likely related to a statistically significant difference in PLT – a parameter used in the calculation of both scores. No association was observed between the values of these indices and liver elastography measurements.

Limitations of the study

This study has several limitations. First, the number of participants enrolled in both the study and control groups was relatively small, and the sample sizes between groups were unequal. Additionally, complete data on some basic laboratory parameters were unavailable for all participants. Another limitation is the absence of liver fibrosis assessment using the diagnostic gold standard – liver biopsy. However, given the invasive nature of this procedure and the lack of clear clinical indications in the study population, its application would have been ethically unjustified. Furthermore, histopathological analysis of intestinal tissue samples to assess fibrosis was not performed, limiting the ability to determine whether reduced FGF-19 levels and elevated CTGF concentrations are directly associated with intestinal fibrosis. An additional limitation of the study is the use of the liver fibrosis indices FIB-4 and APRI, which rely, among other parameters, on PLT – a value that may increase in the presence of inflammation.

Conclusions

Patients with newly diagnosed CD showed no evidence of advanced liver fibrosis at the time of diagnosis, although one patient had liver stiffness values corresponding to F2 according to the METAVIR classification. These findings

suggest that advanced hepatic fibrosis is uncommon at CD diagnosis, while early hepatic involvement cannot be excluded. The observed correlation between elevated elastography values and calprotectin levels may reflect intestinal, rather than hepatic, inflammation. Notably, the decreased concentration of FGF-19 and the increased concentration of CTGF in these patients may be indicative of inflammatory or fibrotic processes occurring within the intestinal wall, rather than in the liver. This interpretation is supported by the absence of liver fibrosis or inflammation in the studied group, pointing toward a gut-specific fibrogenic response. Although noninvasive techniques for liver fibrosis assessment are evolving, none currently match the diagnostic precision of liver biopsy. Longitudinal studies are needed to monitor potential hepatic changes over time, but current findings may emphasize the intestinal origin of biomarker activity in early CD.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.21294419>. The package contains the following files:

Supplementary Fig. 1. Scatter plot illustrating the relationship between PLT and elastography values in the study group.

Supplementary Fig. 2. Scatter plot illustrating the relationship between CRP and elastography values in the study group.

Supplementary Fig. 3. Scatter plot illustrating the relationship between ESR and elastography values in the study group.

Supplementary Fig. 4. Scatter plot illustrating the relationship between ALT and elastography values in the study group.

Supplementary Fig. 5. Scatter plot illustrating the relationship between AST and elastography values in the study group.

Supplementary Fig. 6. Scatter plot illustrating the relationship between GGT and elastography values in the study group.

Supplementary Fig. 7. Scatter plot illustrating the relationship between PLT and FGF-19 in the study group.

Supplementary Fig. 8. Scatter plot illustrating the relationship between CRP and FGF-19 in the study group.

Supplementary Fig. 9. Scatter plot illustrating the relationship between ESR and FGF-19 in the study group.

Supplementary Fig. 10. Scatter plot illustrating the relationship between ALT and FGF-19 in the study group.

Supplementary Fig. 11. Scatter plot illustrating the relationship between AST and FGF-19 in the study group.

Supplementary Fig. 12. Scatter plot illustrating the relationship between GGT and FGF-19 in the study group.

Supplementary Fig. 13. Scatter plot illustrating the relationship between calprotectin and FGF-19 in the study group.

Supplementary Fig. 14. Scatter plot illustrating the relationship between PLT and CTGF in the study group.

Supplementary Fig. 15. Scatter plot illustrating the relationship between CRP and CTGF in the study group.

Supplementary Fig. 16. Scatter plot illustrating the relationship between ESR and CTGF in the study group.

Supplementary Fig. 17. Scatter plot illustrating the relationship between ALT and CTGF in the study group.

Supplementary Fig. 18. Scatter plot illustrating the relationship between AST and CTGF in the study group.

Supplementary Fig. 19. Scatter plot illustrating the relationship between GGT and CTGF in the study group.

Supplementary Fig. 20. Scatter plot illustrating the relationship between calprotectin and CTGF in the study group.

Supplementary Fig. 21. Scatter plot illustrating the relationship between PLT and elastography values in the control group.

Supplementary Fig. 22. Scatter plot illustrating the relationship between CRP and elastography values in the control group.

Supplementary Fig. 23. Scatter plot illustrating the relationship between ALT and elastography values in the control group.

Supplementary Fig. 24. Scatter plot illustrating the relationship between AST and elastography values in the control group.

Supplementary Fig. 25. Scatter plot illustrating the relationship between GGT and elastography values in the control group.

Supplementary Fig. 26. Scatter plot illustrating the relationship between calprotectin and elastography values in the control group.

Supplementary Fig. 27. Scatter plot illustrating the relationship between PLT and FGF-19 values in the control group.

Supplementary Fig. 28. Scatter plot illustrating the relationship between CRP and FGF-19 values in the control group.

Supplementary Fig. 29. Scatter plot illustrating the relationship between ESR and FGF-19 values in the control group.

Supplementary Fig. 30. Scatter plot illustrating the relationship between ALT and FGF-19 values in the control group.

Supplementary Fig. 31. Scatter plot illustrating the relationship between AST and FGF-19 values in the control group.

Supplementary Fig. 32. Scatter plot illustrating the relationship between GGT and FGF-19 values in the control group.

Supplementary Fig. 33. Scatter plot illustrating the relationship between calprotectin and FGF-19 values in the control group.

Supplementary Fig. 34. Scatter plot illustrating the relationship between PLT and CTGF values in the control group.

Supplementary Fig. 35. Scatter plot illustrating the relationship between CRP and CTGF values in the control group.

Supplementary Fig. 36. Scatter plot illustrating the relationship between ESR and CTGF values in the control group.

Supplementary Fig. 37. Scatter plot illustrating the relationship between ALT and CTGF values in the control group.

Supplementary Fig. 38. Scatter plot illustrating the relationship between AST and CTGF values in the control group.

Supplementary Fig. 39. Scatter plot illustrating the relationship between GGT and CTGF values in the control group.

Supplementary Fig. 40. Scatter plot illustrating the relationship between calprotectin and CTGF values in the control group.

Supplementary Table 1. Results of tests to check the assumptions.

Supplementary Table 2. Shapiro–Wilk test results for the study and control groups. All values were considered statistically significant at $p < 0.05$.

Supplementary Table 3. Correlations recalculated using the Kendall coefficient for elastography and laboratory test results in the study group.

Supplementary Table 4. Correlations recalculated using the Kendall coefficient for FIB-4 and laboratory test results in the study group.

Supplementary Table 5. Correlations recalculated using the Kendall coefficient for APRI and laboratory test results in the study group.

Supplementary Table 6. Correlations recalculated using the Kendall coefficient for elastography and laboratory test results in the control group.

Supplementary Table 7. Correlations recalculated using the Kendall coefficient for FIB-4 and laboratory test results in the control group.

Supplementary Table 8. Correlations recalculated using the Kendall coefficient for APRI and laboratory test results in the control group.

Data Availability Statement

The participants in this study did not provide written consent for their data to be shared publicly; therefore, due to the sensitive nature of the research, the supporting data are not available.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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A multidisciplinary diagnostic approach to thoracic endometriosis syndrome: Insights from CT imaging, surgery, and pathology

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Abstract

Background. Thoracic endometriosis syndrome (TES) is the most common extrapelvic manifestation of endometriosis, yet its classification, diagnosis, and treatment remain debated. Thoracic endometriosis syndrome has recently been proposed to be categorized into 3 types: pleural, bronchopulmonary, and diaphragmatic.

Objectives. Our study aims to propose a 3-step approach – computed tomography (CT) imaging, careful intraoperative assessment, and targeted pathology – to enhance the diagnostic process and optimize the management of TES-related pneumothorax.

Materials and methods. We retrospectively analyzed 3 cases of recurrent right-sided catamenial pneumothorax in female patients with a history of pelvic endometriosis, managed surgically at the Department of Thoracic Surgery, Medical University of Gdansk (Poland), between 2021 and 2024. All included patients underwent video-assisted thoracoscopic surgery (VATS), with comprehensive preoperative CT imaging, intraoperative lesion mapping, and postoperative histopathological examination, improving diagnostic accuracy in TES, with a possible impact on therapeutic results.

Results. Chest CT is essential for detecting bullous lesions, aiding in intraoperative identification and surgical treatment. Immunohistochemistry (IHC), assessing estrogen receptor (ER) and CD10 for stromal identification, improves pathological accuracy.

Conclusions. This study underlines the diagnostic value of integrating imaging, intraoperative assessment, and targeted histopathology, as well as clear communication between surgeons, radiologists, and pathologists to ensure accurate diagnosis and an improved therapeutic process for patients in clinical practice.

Key words: Thoracic endometriosis syndrome, catamenial pneumothorax, video-assisted thoracoscopic surgery (VATS), computed tomography imaging, immunohistochemistry

Highlights

- Thoracic endometriosis syndrome (TES) is the most common form of extrapelvic endometriosis, yet it remains difficult to classify and diagnose.
- This study presents a 3-step diagnostic approach comprising computed tomography (CT) imaging, intraoperative identification, and targeted pathological analysis.
- Video-assisted thoracoscopic surgery (VATS), combined with preoperative CT imaging, enhances the surgical evaluation of endometriotic lesions.
- Immunohistochemical staining for estrogen receptor (ER) and CD10 increases diagnostic accuracy.
- Multidisciplinary collaboration among surgeons, radiologists, and pathologists facilitates more precise surgical planning based on accurate imaging, increases the pathological diagnostic yield.

Background

Thoracic endometriosis syndrome (TES) is the most prevalent form of extrapelvic endometriosis,¹ yet it remains surrounded by controversies regarding its classification,² diagnosis,³ and treatment.⁴ Recently, it has been proposed that TES be classified into pleural, bronchopulmonary, and diaphragmatic forms.² The bronchial presentation of TES has rarely been reported, while seeding of endometrial foci on the parietal pleura is exceedingly rare. The most common manifestation of TES, apart from the diaphragmatic form, involves subpleural localization within the pulmonary parenchyma.^{2,5} On this basis, distinguishing between the diaphragmatic and bronchopulmonary forms of TES appears justified and allows for a more appropriate classification of this phenomenon. In pulmonary TES, chest computed tomography (CT) reveals bullous lesions⁵ and solid pulmonary tumors,^{6,7} making CT the primary imaging modality. Magnetic resonance imaging (MRI) is valuable for confirming thoracic endometriosis, offering superior contrast resolution and the ability to characterize hemorrhagic lesions, despite its lower spatial resolution. Magnetic resonance imaging is preferred for visualizing diaphragmatic or pleural implants, while CT scans are crucial for analyzing pulmonary parenchyma, facilitating difficult intraoperative identification and effective surgical treatment.³

Catamenial pneumothorax represents a distinct clinical manifestation within the spectrum of TES. Although it is likely not the most prevalent form of TES, it constitutes a clinically significant cause of emergency hospital admissions related to primary pneumothorax and, in particular, recurrent pneumothoraces in women of reproductive age.⁸ The mean age at diagnosis of TES typically ranges between 34 and 37 years,⁹ which is generally attributed to the long-standing, often undiagnosed or inadequately treated course of pelvic endometriosis preceding thoracic involvement in 85% of patients.¹⁰ Management of patients with catamenial pneumothorax remains challenging, largely due to an exceptionally high recurrence rate, reported to reach or even exceed 40% despite standard surgical

interventions.^{11,12} These unfavorable outcomes underscore the need to reconsider the conventional approach to such patients, as catamenial pneumothorax should not be managed as a form of spontaneous pneumothorax. Instead, it has been proposed that TES requires treatment within dedicated multidisciplinary teams integrating thoracic surgery, gynecology, radiology, pathology, and hormonal management to optimize diagnostic accuracy and reduce the risk of recurrence.¹³

Adequate pathological analysis of TES involves immunohistochemistry (IHC) to evaluate the expression of estrogen receptor (ER) and CD10 for stromal identification. Typically, the examination of material resected during pneumothorax surgery does not include ER IHC for endometrial gland visualization or CD10 IHC for stromal visualization.^{14,15} Although hematoxylin and eosin (H&E) staining may be indicative in large pelvic endometriomas, it is often insufficient for the minimal lesions typically found in pulmonary TES.

Objectives

We propose a 3-step approach for patients with TES-related pneumothorax: comprehensive CT assessment, meticulous intraoperative visualization, and dedicated pathological evaluation.

Materials and methods

This study is a retrospective analysis of 3 patients who underwent surgery for recurrent right-sided catamenial pneumothorax at the Department of Thoracic Surgery, Medical University of Gdansk, Poland, between February 25, 2021, and January 22, 2024. Each patient had a documented history of pelvic endometriosis treatment. We reanalyzed data from preoperative chest CT imaging, intraoperative findings, and postoperative examination of surgical specimens. The study was approved by the Institutional Review Board of the Medical University

of Gdansk (approval No. NKBBN/134/2020-2021 issued on October 15, 2021). The study was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. The approval covered the retrospective analysis of data routinely collected in the electronic medical records prior to the date it was issued; the data of the 1st patient were therefore included under the approved protocol. Individual patient consent was not required owing to the retrospective, observational design of the study.

Computed tomography scans were performed before the surgical intervention. For patients A and B, preoperative scans were performed 1 month before surgery, while for patient C, the scan was performed 1 week prior. The phase of the menstrual cycle was not recorded at the time of imaging. Patients A, B, and C had experienced 4, 2, and 2 prior recurrences of right-sided catamenial pneumothorax, respectively. All patients subsequently underwent right-sided video-assisted thoracoscopic surgery (VATS).

The standardized VATS approach included positioning the patient in the left lateral decubitus position, utilizing double-lumen intubation with one-lung ventilation, and making incisions as follows: a port in the 7th intercostal space in the anterior axillary line (30° scope), a 3-cm utility incision in the 5th intercostal space with a soft tissue retractor, and a 2nd 10-mm incision in the 8th intercostal space in the posterior axillary line without a port.

During the procedure, a thorough assessment of the parietal and visceral pleura, lung, and diaphragm was conducted. Any bullous lesions were excised, and diaphragmatic defects were either reconstructed or partially excised. The procedure concluded with a complete pleurectomy and placement of a 24-French (F) chest drain. Detailed tissue-processing and immunohistochemical staining protocols are provided in Supplementary File 1.

Microscopic assessment of the slides was performed by 2 pathologists (A.C. and R.P.). A positive ER staining result was indicated by a nuclear reaction, while a positive CD10 staining result was identified by a membrane reaction.

Published case reports emphasize the role of ER and CD10 staining in histopathological confirmation of TES, whereas progesterone receptor (PR) immunostaining appears to be less frequently reported.¹⁶ However, recent studies recommend including PR alongside ER and CD10, as it may improve recognition of ectopic endometrial foci in the chest cavity. Given that the PR staining was not performed in our study, we suggest that routine PR assessment may be useful in the diagnosis of suspected TES.

A positive pathological diagnosis of pulmonary endometriosis was retrospectively compared with intraoperative findings and preoperative imaging. A pattern of consecutive preoperative, intraoperative, and postoperative lesions typical of pulmonary endometriosis was presented.

Results

Three female patients of reproductive age (37, 39, and 43 years) with recurrent catamenial right-sided pneumothorax participated in this study. The primary symptom for all patients was pleuritic pain on the right side of the chest. Preoperative CT scans (axial, frontal, and sagittal views) revealed bullous lesions in the middle lobe of the right lung in all patients (Fig. 1).¹⁷ Every patient presented with solitary, non-clustered small bullous lesions on CT imaging, which were round and subcentimeter in patients A and B and elliptical, measuring 20 mm, in patient C. No additional lesions were observed. Using VATS, we identified bullous lesions in the middle lobe of the right lung in all 3 patients (Fig. 2), diaphragmatic defects with nodules in 2 patients, and a porous area without nodules in 1 patient.

During VATS, all patients underwent wedge excision of pulmonary bullous lesions and complete pleurectomy. Two patients had partial resection of the tendinous part of the diaphragm without mesh, and 1 patient had direct diaphragm reconstruction. Clinical details are summarized below in Table 1. Histopathological examination of tissue samples obtained intraoperatively from the right lung revealed subpleural emphysematous changes. Immunohistochemical evaluation of endometrial lesions used antibodies specific for ER α and CD10, revealing estrogen receptor-positive (ER+) cells characteristic of endometrial glands and CD10+ cells indicating the presence of endometrial stroma (Fig. 3).

In patient A, pleural tissue fragments showed 8 subpleural microfoci of endometriosis measuring 0.5–3 mm in diameter, composed of endometrial stromal cells (CD10+, ER+) accompanied by dilated blood vessels and small hemorrhagic foci. In patient B, a peripheral lung parenchyma specimen contained a single subpleural microfocus of endometriosis measuring 1.5 mm, characterized by numerous endometrial stromal cells (CD10+, ER+), dilated blood vessels, and deposits of anthracotic pigment. In patient C, pleural tissue fragments revealed a single subpleural microfocus of endometriosis up to 2.5 mm in size, composed mainly of endometrial stromal cells (CD10+, ER+) and associated with dilated blood vessels.

The operative times for patients A, B, and C were 75, 70, and 139 min. The postoperative course was uncomplicated in all patients, with hospital stays of 4, 6, and 4 days, respectively. Patients A and B had not received hormonal therapy before admission. After hospitalization, patient A received dienogest, and patient B received combined therapy with dienogest and estradiol. Patient C was already on hormonal therapy, receiving dienogest at admission. Postoperative hormonal management was determined individually by the attending physician. Patient A experienced 1 right-sided episode of recurrent catamenial pneumothorax 45 months after surgery, during a 50-month follow-up. Imaging confirmed the recurrence, but no surgical treatment or chest drainage was required, and no

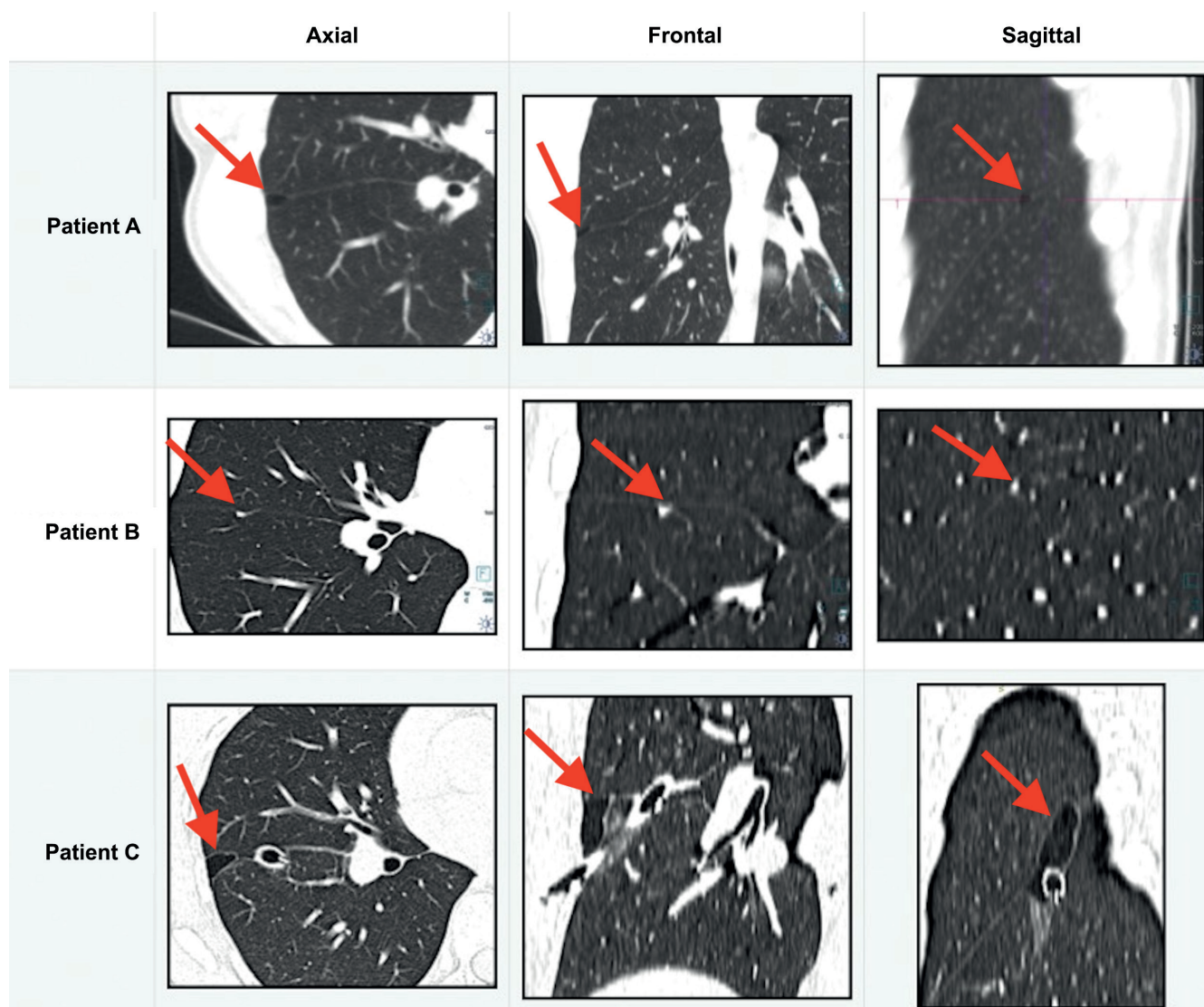


Fig. 1. Computed tomography (CT) scans (axial, frontal, and sagittal) of all 3 patients (A, B, and C) showing bullous lesions in the middle lobe of the right lung (red arrows)

pathology was detected on the left side. Patients B and C have not experienced recurrence in 26 and 15 months of follow-up, respectively.

Discussion

This study underscores the necessity for TES-focused preoperative and postoperative assessments to improve the diagnosis of TES. Patients with recurrent TES-related pneumothoraces and underdiagnosed pulmonary TES are common, largely due to low awareness of TES among thoracic care teams. Currently, there is no standardized approach for patients with catamenial, TES-related pneumothorax. Thoracic endometriosis syndrome remains an orphan disease and a rare but probably important cause of recurrent pneumothoraces. Its management warrants a modified and highly coordinated approach, beginning with tailored radiological evaluation, followed by meticulous

surgical assessment, comprehensive pathological examination, and, finally, postoperative guidance on hormonal therapy. Although it is not yet established whether this condition should be managed exclusively in high-volume centers, it is clear that patients with endometriosis benefit from a multispecialist model of care involving gynecological and fertility counseling, pain management, general surgical and urological input, specialized physiotherapy, dietary support, psychological and psychiatric care, and access to patient support groups.¹⁸ Thoracic endometriosis syndrome represents a rare and distinctive manifestation within this disease spectrum, and in cases of recurrent symptoms, referral to a multidisciplinary center should be strongly considered.¹³

Catamenial pneumothorax should be considered in ovulating women presenting with spontaneous pneumothorax, even when there is no clinical history suggestive of pelvic endometriosis. Video-assisted thoracoscopic surgery is currently considered the gold standard for the management of TES-related pneumothorax, as it allows thorough

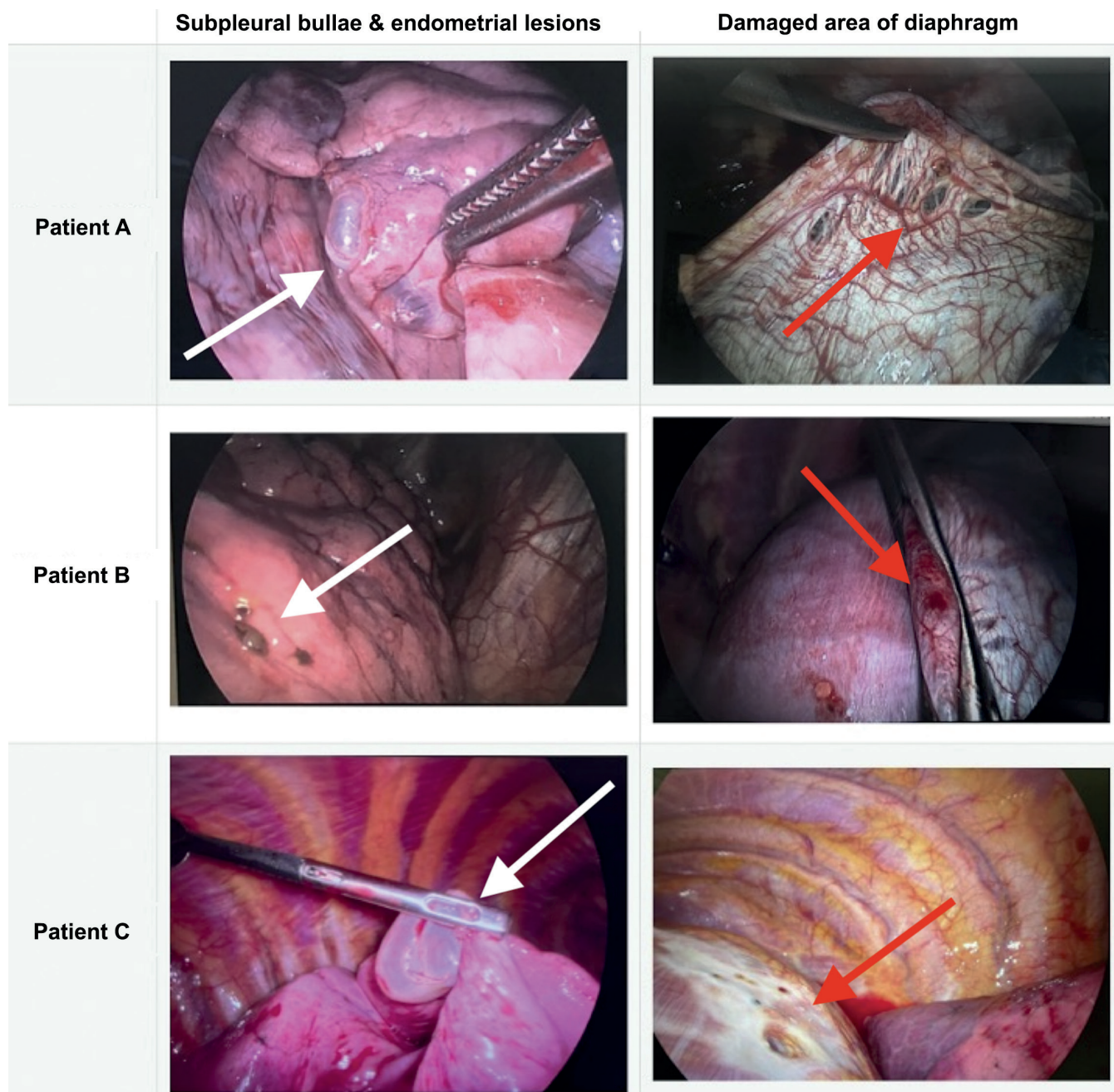


Fig. 2. Surgical findings in all 3 patients (A, B, and C) – subpleural lesions (white arrows) and a damaged diaphragm (red arrows)

inspection of the pleural cavity, lung parenchyma, and diaphragm, together with definitive surgical treatment using a minimally invasive approach.² In cases with suspected diaphragmatic involvement, the addition of simultaneous laparoscopic visualization may be advantageous,^{13,19} since assessment of diaphragmatic lesions from the pleural surface alone may underestimate the true extent of disease affecting the diaphragmatic muscle and its peritoneal aspect.^{20,21}

Several surgical series emphasize the importance of a meticulous intraoperative assessment, particularly careful inspection of the diaphragmatic surface during video-assisted thoracic procedures.¹² Limited diaphragmatic interventions, such as plication of suspicious areas, have been reported to be effective in selected cases, while

adjunctive postoperative hormonal suppression has been shown to improve outcomes, especially in more complex or recurrent presentations. Collectively, published data indicate that diaphragmatic involvement is detected in a substantial proportion of patients, and that outcomes vary according to the extent and method of diaphragmatic management. Direct surgical repair involving mesh-based reconstruction has been described, yet analyses expressed in proportional terms consistently suggest superior results when all visible diaphragmatic abnormalities are actively addressed.⁹ In particular, reconstruction with prosthetic materials, including biological meshes, has been associated with favorable perioperative safety and durable long-term results when compared with more limited approaches.

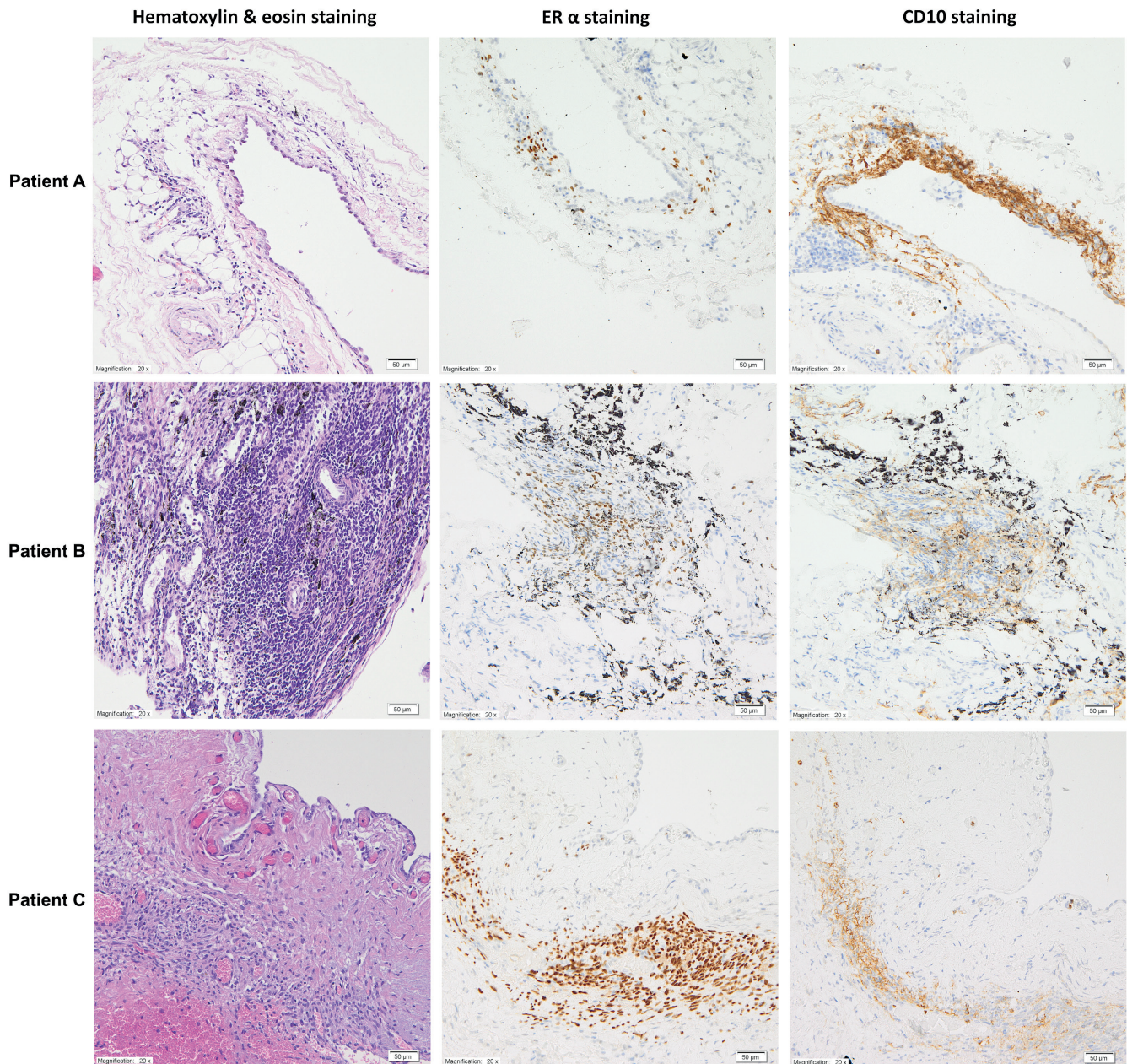


Fig. 3. Hematoxylin and eosin (H&E) staining and immunohistochemical (IHC) staining for estrogen receptor alpha (ER-α) and CD10 in specimens from patients A, B, and C

Large literature-based series further demonstrate that optimal management of catamenial pneumothorax and other thoracic manifestations of endometriosis relies on a combined surgical and hormonal strategy. Surgical treatment commonly encompasses apical lung resection, excision of all suspected visceral and parietal pleural lesions, and partial resection or reconstruction of the diaphragm in the presence of nodules or fenestrations, aiming to eliminate diseased tissue and restore diaphragmatic integrity. Obliteration of the pleural cavity (pleurectomy, surgical abrasion, and chemical or talc pleurodesis) is considered an important component of treatment, intended to reduce the risk of recurrence related to microscopic or newly implanted endometrial foci.²² Despite these observations, no uniform, evidence-based recommendations

currently exist regarding the optimal extent of surgery. Nevertheless, based on the cumulative evidence from the literature and our own experience, we advocate a comprehensive approach consisting of removal of all suspected pulmonary lesions, including small or atypical bullous lesions, systematic repair or reconstruction of the diaphragm, and adjuvant hormonal therapy following thoracic surgery.² Female patients of reproductive age with pneumothorax, particularly right-sided, should be thoroughly evaluated for a TES-related etiology prior to any treatment, including chest drainage.

The risk of TES increases in the presence of coexisting pelvic endometriosis,^{13,23} the recurrent nature of the pneumothorax,^{24,25} a history of infertility,^{26,27} and menses-related arm or chest pain.¹⁹ The incidence of TES-related

Table 1. Clinical characteristics of all patients

Clinical aspect	Patient A	Patient B	Patient C
Age [years]	37	39	43
Primary reason for hospital admission	Recurrent catamenial right-sided pneumothorax	Recurrent catamenial right-sided pneumothorax	Recurrent catamenial right-sided pneumothorax
Main symptom	Pleuritic pain on the right side of the chest	Pleuritic pain on the right side of the chest	Pleuritic pain on the right side of the chest
Previous diagnosis of endometriosis	Yes – status post resection of endometrial lesions in the lesser pelvis	Yes – status post surgical treatment of ovarian endometrial cysts	Yes – status post resection of an endometrial lesion in the abdominal cavity
Computed tomography findings	Bullous lesion in the middle lobe of the right lung	Bullous lesion in the middle lobe of the right lung	Bullous lesion in the middle lobe of the right lung
Surgical treatment	Wedge resection of the bullous lesion in the middle lobe; reconstruction of the diaphragm; pleurectomy	Wedge resection of lesions in the right lung; partial resection and reconstruction of the diaphragm; pleurectomy	Wedge resection of the bullous lesion in the middle lobe; partial resection and reconstruction of the diaphragm; pleurectomy
VATS – intraoperative findings	Emphysematous bullous lesion in the middle lobe of the right lung; defect of the diaphragm	Emphysematous bullous lesion in the middle lobe of the right lung; defect of the diaphragm with nodules	Emphysematous bullous lesion in the middle lobe of the right lung; defect of the diaphragm with nodules
Operative time [min]	75	70	139
Postoperative stay [days]	4	6	4
Hormonal therapy at admission	None	None	Dienogest
Hormonal therapy after surgical treatment	Dienogest initiated after the procedure and continued for 15 months	Combined therapy with dienogest and estradiol initiated after surgery and maintained for 26 months	Dienogest therapy was continued for 15 months
Recurrences of catamenial pneumothorax	One episode of recurrent catamenial pneumothorax	None	None

VATS – video-assisted thoracoscopic surgery.

pneumothorax among all patients of childbearing age with pneumothorax ranges from 7% to 37%.²⁸ In such cases, patients should be referred to centers capable of complex interventions, where multidisciplinary care – involving specialists in thoracic surgery, gynecology, radiology, pathology, anesthesia, and pain management – is readily available, and where surgical teams have the expertise to perform combined procedures, including simultaneous thoracoscopic and laparoscopic evaluation of the diaphragm to ensure comprehensive identification and resection of all endometriotic foci.¹³ The need for dedicated TES care teams remains an open question, though this solution has been discussed by a specialist team organized by the Polish Ministry of Health.^{29,30} As a general principle, surgery for endometriosis should be meticulously planned with the aim of identifying and removing all foci of deep infiltrating endometriosis (DIE).³¹ In thoracic endometriosis, diaphragmatic involvement is present in approx. 84% of cases, and VATS is the preferred surgical approach in the majority of patients, while laparoscopy is performed concomitantly or in a staged fashion in about half of them. Taken together, these observations support the need to plan for complete management of all diaphragmatic endometriotic foci, including direct suturing of defects or ablation of superficial lesions, and excision of diaphragmatic segments in cases of DIE with subsequent reconstruction, preferably with mesh implantation.^{16,32}

There are various interpretations of the radiological presentation of pulmonary TES.^{3,5,7} In our view, MRI may be beneficial for identifying diaphragmatic implants, whereas CT remains the standard for strictly pulmonary lesions. Computed tomography is useful for diagnosing extensive diaphragmatic defects that can lead to the rarely observed occurrence of hepatic herniation.³³ In our series, all patients had diaphragmatic defects identified intraoperatively, despite the absence of a preoperative diagnosis on CT scans. A meticulous preoperative CT assessment with careful attention to small, subpleural bullous lesions is indicated, as these subtle findings may represent early or atypical manifestations of thoracic endometriosis rather than incidental or clinically insignificant small bullous lesions. Although their predominant occurrence in the middle lobe may be coincidental, this pattern could also reflect the inherently unpredictable distribution of subpleural endometriotic foci. Whereas previous studies have primarily described CT and MRI as diagnostic methods for evaluating abdominal and diaphragmatic endometriotic lesions,³ our study highlights the value of CT in identifying endometrial features within the thoracic cavity itself by presenting 3 cases in which pulmonary lesions were delineated on CT, thereby reinforcing the diagnostic value of meticulous thoracic CT evaluation.⁵ Recognizing these nuances can improve diagnostic accuracy and guide more targeted intraoperative assessment. Unlike previous

studies, which associated TES-related pneumothorax with solid tumors,^{3,7} our research identified minimal subpleural bullous lesions that correlate with the pathological presentation of TES. These subtle intrapulmonary bullous lesions are adequately visualized with non-contrast CT. Large lung cancer screening trials using CT have improved the understanding of sub-centimeter solid pulmonary nodules. The clinical interpretation of these lesions, found in nearly 25% of screened individuals,³⁴ is challenging. Most subpleural nodules are benign, often attributed to carbon-loaded lymph nodes.³⁵ The CT scans of patients A and B did not reveal significant bullous lesions or other pathological findings at first glance; however, on retrospective review, subtle abnormalities can be appreciated, underscoring the need for meticulous radiologic assessment in premenopausal patients with recurrent pneumothorax, in whom TES should be considered. This radiologic impression was confirmed intraoperatively, when patient C was found to have the most substantial disease burden, whereas patients A and B demonstrated only minor lesions. We strongly advocate for the preoperative identification of even minimal bullous lesions in patients with suspected TES-related pneumothorax, given the high risk of their association with TES.

In many articles addressing IHC in thoracic endometriosis, antibodies for detecting CD10 are predominantly utilized, often in conjunction with antibodies for ER and/or PR detection. While CD10 is generally adequate for identifying endometriosis, its co-expression with ER/PR can be beneficial in diagnosing catamenial pneumothorax.^{14,15} Indeed, ER, PR, and CD10 are the most commonly evaluated markers in TES. However, interferon-inducible transmembrane protein 1 (IFITM1) for stromal cells and PAX8 for epithelial cells have also been proposed as sensitive markers in extrapelvic endometriosis.¹⁴ In a retrospective study involving 55 women undergoing surgery for pneumothorax, IHC for ER, PR, and CD10 was performed on bland stromal spindle cells of the lung.³⁶ A total of 37 cases that tested positive for both receptors were subsequently classified as exhibiting either “scattered” or “aggregated” patterns. The scattered pattern (33/37) was CD10-negative and associated with a lower 5-year recurrence rate of pneumothorax (16.7%), whereas the aggregated pattern (4/37) was CD10-positive and linked to a higher 5-year recurrence rate (62.5%). Notably, as the scattered pattern was also found in the male control group, a diagnosis of thoracic endometriosis was made only in cases exhibiting the aggregated pattern.³⁶ All patients in our study were ER- and CD10-positive. Although further research is needed, meticulous evaluation of staining patterns may enhance the precision of TES diagnosis. Early identification of even subtle lesions appears to contribute to a more favorable prognosis, reflected in fewer recurrences of TES-related pneumothorax. Close collaboration between surgical and pathological teams remains essential for recognizing lesions most likely associated with TES. Importantly, we believe that TES should be considered a potential etiology

of recurrent pneumothorax in every woman of premenopausal age. Diagnosing endometriotic foci within the lung not only establishes the presence of thoracic endometriosis but also enables appropriate systemic treatment of endometriosis as a disease entity. Incorporating routine ER/PR/CD10 immunostaining into the pathological assessment of lung specimens obtained during pneumothorax surgery in premenopausal women may further increase diagnostic yield and improve overall quality of care, ultimately helping to reduce recurrence rates. Although simple pneumothorax surgery can be effectively performed using a uniportal VATS approach, our strategy prioritizes a more comprehensive intraoperative assessment, including meticulous inspection of the lung parenchyma, diaphragmatic evaluation, partial resection, and, when necessary, diaphragmatic reconstruction. While increasingly complex procedures are now performed through uniportal access in high-volume centers of excellence, a utility incision complemented by 1 or 2 additional ports remains the standard technique in many institutions and continues to be widely adopted in routine clinical practice.³⁷ Moreover, we believe that the debate regarding the optimal number of ports in minimally invasive thoracic surgery has largely diminished following the widespread introduction of robot-assisted approaches.

Limitations of the study

This study acknowledges several important limitations. First, it involves a small cohort of patients treated at a single center by 1 general thoracic surgeon. While this may reflect the clinical approach of a single specialist, such scenarios are not uncommon in the context of orphan diseases. Second, the order in which assessments were performed represents a limitation. Ideally, a meticulous radiological assessment should precede intraoperative findings, which are then confirmed by a pathologist’s report. In our study, intraoperative findings, when confirmed by the pathological report, were subsequently compared with preoperative CT scans, which identified minimal yet clinically significant lesions. Third, the follow-up duration was variable among patients A, B, and C (50, 26, and 15 months). Moreover, data on the menstrual cycle phase at the time of chest CT imaging were not collected. Although some recent studies have assessed the radiological features of TES in relation to the menstrual cycle phase, this approach is not routinely implemented. The absence of this information may have limited the interpretative specificity of our imaging findings. We suggest that future studies may benefit from standardized documentation of menstrual cycle phase at the time of imaging. An additional potential limitation is the absence of PR staining, which may have reduced our ability to detect or confirm some TES lesions. Despite all these limitations, we assert that it is both clinically and scientifically justified to emphasize the recommendation not to underestimate the risk of TES in small, subpleural bullous lesions located outside the lung apex.

Conclusions

Small subpleural bullous lesions may represent radiological manifestations of TES. Preoperative CT scans likely facilitate the intraoperative localization of minimal lesions. Effective communication between surgeons, radiologists, and pathologists enhances the diagnostic yield in TES, potentially influencing treatment outcomes.

Supplementary data

The supplementary material is available at <https://doi.org/10.5281/zenodo.17641838>. The package contains the following files:

Supplementary File 1. Tissue processing and analysis.

Data Availability Statement

The datasets supporting the findings of the current study are available from the corresponding author on reasonable request. The data are not publicly available due to ethical reasons, by reason of human research participant data presence.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Prospective analysis of medical conditions and interventions among athlete and non-athlete groups at 2 major international multi-sport tournaments

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Abstract

Background. Medical preparedness for major international multi-sport tournaments requires effective planning of venue medical services for athlete and non-athlete populations. Understanding differences in medical intervention patterns is essential for resource allocation.

Objectives. To compare on-site medical interventions, hospital transports, and related medical conditions managed by venue medical teams (VMTs) between athlete and non-athlete populations during 2 international multi-sport tournaments, and to identify sport disciplines associated with higher hospital transport rates among athletes.

Materials and methods. Prospective data from the World Games 2017 and the European Games 2023, both held in Poland, were analyzed. The study included 9,672 athletes and 355,215 non-athletes. Interventions were classified as on-site medical interventions and hospital transports. Medical conditions were classified as injuries or illnesses and as mild or severe. Incidence rates per 1,000 participant-days and event rates per 100 participants were calculated.

Results. A total of 1,021 medical interventions and 114 hospital transports were recorded. Compared with non-athletes, athletes demonstrated substantially higher incidence rates across all analyzed outcomes. The pooled incidence rate ratio (IRR) was 5.88 (95% confidence interval (95% CI): 5.19–6.65) for total medical interventions and 16.69 (95% CI: 11.24–24.77) for hospital transports, with the largest between-group differences observed for injury-related medical interventions (IRR = 10.87, 95% CI: 8.76–13.49) and injury-related hospital transports (IRR = 24.44, 95% CI: 15.30–39.05). Mild conditions predominated in both groups, whereas injury-related conditions accounted for a greater proportion of medical interventions among athletes and illness-related conditions among non-athletes. Hospital transports were uncommon in both groups but occurred substantially more frequently among athletes and were predominantly injury-related. Muaythai, American football, BMX freestyle cycling, sumo, and beach handball had the highest hospital transport rates.

Conclusions. Athletes and non-athletes demonstrated distinct patterns of medical interventions and related medical conditions during major international multi-sport tournaments. Combined surveillance of both populations provides a comprehensive assessment of VMT workload and supports evidence-based medical planning for future tournaments.

Key words: mass gathering medicine, hospital transport, major sporting tournament, on-site medical intervention

Highlights

- Athletes had substantially higher rates of medical interventions and hospital transports than non-athletes during international multi-sport tournaments.
- Injury-related medical interventions and hospital transports were markedly more frequent among athletes than non-athletes.
- Mild medical conditions predominated, while athletes experienced proportionally more injury-related conditions and non-athletes more illness-related conditions.
- Muaythai, American football, BMX freestyle cycling, sumo, and beach handball showed the highest hospital transport rates among athletes.
- Combined athlete and non-athlete medical surveillance supports evidence-based venue medical planning and resource allocation at major sporting events.

Background

Medical preparedness for major sport tournaments (MSTs) is designed to ensure the health and safety of all participants.^{1–4} Effective medical planning requires an integrated model, considering all groups involved – participating athletes and non-athletes, including spectators, technical staff, and volunteers.^{5–10} Unique challenges at multi-sport MSTs include dispersed venues, tight competition schedules, diverse sport disciplines, high participant mobility, and constrained medical resources.^{6,7,9,10} Comprehensive risk analyses therefore incorporate tournament- and venue-specific characteristics, emergency medical system (EMS) and healthcare system capacities, public health, and crisis management.^{7–10} Medical data from previous tournaments support precise venue planning.^{1,8–11} Athlete-specific surveillance data on injuries and illnesses have been analyzed across the most prominent tournaments worldwide.^{12–23} The recently published meta-analysis of 27 articles showed that the overall incidences of injuries and illnesses during the Olympic Games (OG) were 6.50 (95% confidence interval (95% CI): 5.91–7.15) and 3.62 (95% CI: 2.83–4.65), respectively.¹² The highest incidences of injuries were observed in taekwondo, football, boxing, handball, and weightlifting. Lower limb injuries had the highest incidence among injury-related medical conditions.¹² For non-athlete groups, evidence from prior MSTs demonstrates low rates of on-site medical interventions, mostly related to weather conditions, with only modest increases in emergency department workload.^{18–24} The majority of medical conditions have been mild and managed on-site, with only a small proportion requiring hospital transport.^{17–24}

Despite the growing number of international multi-sport MSTs, comparative analyses evaluating medical service utilization across different types of multi-sport events remain scarce. Poland hosted 2 MSTs within a 6-year period: the World Games (WG) 2017 and the European Games (EG) 2023. Both tournaments were held in similar geographical, environmental, and healthcare policy

conditions, with comparable medical reporting systems. This unique setting provides an opportunity to compare patterns of medical service utilization among athletes and non-athletes across different multi-sport MSTs.

Objectives

The primary objective was to analyze whether distinct patterns existed in medical interventions and hospital transport requirements managed by the venue medical teams (VMTs) among athlete and non-athlete populations during 2 international multi-sport MSTs. The study sought to compare their incidence rates (IRs) and patterns between athlete and non-athlete populations and between tournaments. The secondary objective – within the athlete cohort – was to identify sport disciplines associated with a higher frequency of hospital transports and to determine related medical conditions.

Materials and methods

World Games 2017 was a global tournament featuring non-Olympic disciplines. A total of 3,292 athletes competed in 26 indoor and outdoor venues over 11 days. Table 1 lists the disciplines and participants. The tournament was supported by 7,306 staff and volunteers and attracted an estimated 163,324 spectators, based on ticket distribution records. The local organizing committee distributed tickets, ensuring a primarily local audience. The host country provided medical services for all participants. The medical team included 480 medical staff, up to 40 ambulances per day, and 4 referral hospitals. No team physicians were officially registered.

European Games 2023 was a European tournament comprising 10 non-Olympic and 19 Olympic disciplines. A total of 6,380 athletes competed in 26 indoor and outdoor venues over 12 days. Table 2 lists the disciplines and participants. The event was supported by approx. 5,000 staff and

Table 1. World Games 2017 disciplines and athletes

Discipline	NOC						Total	Competition days
	Africa (n = 12)	Asia (n = 28)	Europe (n = 42)	North America (n = 10)	Oceania (n = 3)	South America (n = 8)		
Air sports	1	6	41	10	5	1	64 (1.9%)	4
American football	0	0	140	40	0	0	180 (5.5%)	2
Archery	5	10	60	14	5	2	96 (2.9%)	8
Beach handball	22	20	65	0	20	40	167 (5.1%)	4
Billiards	4	17	30	8	3	2	64 (1.9%)	5
Boules	7	12	61	2	1	12	95 (2.9%)	3
Bowling	0	20	24	12	0	8	64 (1.9%)	4
Canoe polo	0	8	80	8	16	0	112 (3.4%)	3
Dance sports	2	20	100	8	4	12	146 (4.4%)	2
Finswimming	0	17	74	0	0	8	99 (3.0%)	2
Fistball	0	0	31	0	0	29	60 (1.8%)	4
Floorball	0	0	70	14	0	0	84 (2.6%)	4
Flying disc	0	14	14	28	14	14	84 (2.6%)	3
Gymnastics	6	63	188	19	10	9	295 (9.0%)	6
Indoor rowing	4	7	44	3	0	1	59 (1.8%)	2
Ju-jitsu	5	19	93	3	0	7	127 (3.9%)	2
Karate	11	17	48	4	7	9	96 (2.9%)	2
Kickboxing	11	13	59	8	0	5	96 (2.9%)	2
Korfball	0	28	70	0	14	0	112 (3.4%)	5
Lacrosse	0	15	30	30	15	0	90 (2.7%)	4
Life saving	0	20	60	0	20	0	100 (3.0%)	2
Muaythai	3	24	53	4	1	3	88 (2.7%)	2
Orienteering	0	4	64	4	4	4	80 (2.4%)	3
Powerlifting	2	11	41	14	0	12	80 (2.4%)	3
Roller sports	0	14	135	37	3	47	236 (7.2%)	10
Speedway	0	0	18	0	3	0	21 (0.6%)	1
Sport climbing	2	12	44	5	2	1	66 (2.0%)	3
Squash	3	8	40	3	4	3	61 (1.9%)	4
Sumo	6	21	40	10	4	14	95 (2.9%)	2
Tug of war	11	20	164	0	0	0	195 (5.9%)	3
Waterski and wakeboard	0	7	47	15	5	6	80 (2.4%)	4
Total*	105 (3.2%)	447 (13.6%)	2028 (61.6%)	303 (9.2%)	160 (4.9%)	249 (7.6%)	3292 (100%)	11

NOC – National Olympic Committee. *total number of athletes represents the sum across all disciplines; total competition days refer to the overall tournament duration.

8,000 volunteers and attracted an estimated 171,585 spectators, primarily from the local area. Because of dispersed tournament locations, local medical staff included several hundred professionals from 5 medical service providers, with 7 local medical coordinators, 11 referral hospitals, and 5 ambulatory care units. Lifeguard and mountain rescue teams were additionally deployed for water and mountain sports. National team physicians provided medical support for athletes, and local medical services provided support and transport to hospitals.

A standardized medical reporting system, tailored to the tournament settings, was implemented for both

tournaments. Medical coordinators recorded anonymized medical data from VMTs daily and aggregated it into reports. Figure 1 shows an example of the daily report form. Data from team physicians were included only when local medical support was requested, including all hospital transports. Surveillance was based on the standardized metrics, including on-site medical interventions and hospital transports.^{25–27}

Medical conditions were grouped into injuries and illnesses, mild and severe. The definitions of the International Olympic Committee (IOC) were adopted: an injury is tissue damage or other disarrangement of normal physical

Table 2. European Games 2023 disciplines and athletes

Discipline	NOC	Athletes	Competition days
Archery	36	124 (1.9%)	7
Athletics	N/A	1671 (26.2%)	6
Badminton	36	141 (2.2%)	7
3×3 basketball	19	128 (2.0%)	4
Breaking	15	32 (0.5%)	2
Cycling BMX freestyle	19	47 (0.7%)	2
Boxing	43	319 (5.0%)	9
Beach soccer	10	168 (2.6%)	5
Climbing	18	89 (1.4%)	4
Canoe slalom	29	180 (2.8%)	4
Canoe sprint	35	277 (4.3%)	4
Diving	25	126 (2.0%)	7
Fencing	39	464 (7.3%)	6
Beach handball	10	188 (2.9%)	3
Judo	22	213 (3.3%)	1
Kickboxing	22	122 (1.9%)	3
Karate	33	96 (1.5%)	2
Modern pentathlon	25	118 (1.8%)	6
Cycling mountain bike	25	131 (2.1%)	3
Muaythai	23	79 (1.2%)	3
Padel	24	126 (2.0%)	5
Rugby sevens	15	311 (4.9%)	3
Shooting	45	387 (6.1%)	7
Ski jumping	15	93 (1.5%)	5
Artistic swimming	25	170 (2.7%)	5
Tegball	21	68 (1.1%)	4
Taekwondo	44	248 (3.9%)	4
Triathlon	28	112 (1.8%)	3
Table tennis	32	152 (2.4%)	9
Total*	48	6380 (100%)	12

NOC – National Olympic Committee. *total number of athletes represents the sum across all disciplines; total NOCs and competition days refer to the entire tournament.

function due to participation in sports (for athletes) or tournament activities (for non-athletes), resulting from rapid or repetitive transfer of kinetic energy; an illness is a complaint or disorder not related to injury. Severe conditions were those resulting in prolonged absence from participation or requiring hospital transport, whereas mild conditions did not significantly restrict health.²⁷ A statement was obtained from the Bioethics Committee at the Institute of Mother and Child in Warsaw (Poland), confirming that this analysis did not require individual consent.

The analyzed outcomes included on-site medical interventions and hospital transports related to injuries and illnesses recorded during the analyzed MSTs in both athlete and non-athlete groups. The primary outcomes were total medical interventions and total hospital transports

provided by the VMTs. Secondary outcomes included injuries and illnesses, and mild and severe medical conditions. Secondary analyses were considered exploratory and interpreted primarily on the basis of incidence rate ratios (IRRs) and corresponding 95% CIs.

Outcome measures included incidence rates per 1,000 participant-days and event rates per 100 participants, each with 95% CIs. Participant-days were calculated as the total days of exposure contributed by all participants. For athletes, participant-days were estimated by multiplying the number of athletes in each discipline by the number of official competition days. For staff members and volunteers, participant-days corresponded to their working days, whereas spectator exposure was estimated from distributed single-day tickets, with each ticket representing 1 spectator-day. Event rates were compared using Poisson regression models with the logarithm of participant-days included as an offset. Incidence rate ratios with 95% CIs were estimated from model coefficients. Athletes and non-athletes were compared using pooled data from both tournaments, while tournaments were compared separately for athletes and non-athletes.

Because only 2 tournaments were available, clustering at the tournament level could not be evaluated reliably; therefore, tournaments were compared directly using Poisson IRRs. Sparse event counts, particularly illness-related hospital transports, were interpreted cautiously, and exact Poisson methods were considered where appropriate. Statistical inference was based primarily on effect estimates and 95% CIs. Associations were considered statistically significant when the 95% CI did not include 1.0. All analyses were performed in Microsoft Excel 2021 Professional Plus (Microsoft Corp., Redmond, USA), with additional validation in R v. 4.5.0 (R Foundation for Statistical Computing, Vienna, Austria).

The analysis was based on complete surveillance data and did not involve sampling of participants or medical events. Nevertheless, medical events were considered realizations of underlying stochastic processes. Incidence measures, 95% CIs, and IRRs were therefore calculated to quantify variability and enable standardized comparisons between tournaments and participant groups. These measures are not intended to support inference to the general population of athletes or non-athletes but to characterize uncertainty in event occurrence and observed differences.

Results

The cohort comprised nearly 365,000 participants – 9,672 athletes and 355,215 non-athletes. A total of 1,021 medical interventions and 114 hospital transports were reported. Table 3 presents the distribution of medical condition categories among medical interventions and hospital transports by group and tournament. There were 116 venue reports and 15 daily reports at WG 2017, and 134 venue reports and 16 daily reports at EG 2023,

Daily Medical Report					
date		locations		sport disciplines	
medical staff	ALS Ambulance			on-site physicians	
	BLS Ambulance			on-call physicians	
	First Aid Room			physicians - total	
	paramedics			paramedics - total	
medical interventions	group	on-site medical interventions	transport to hospital	description of medical conditions	
	athletes				
	spectators				
	technical staff				
	volunteers				
	total				
deaths	number		description		
nationality of hospitalized foreigners					
identified or suspected public health risk			description		
other relevant conditions			description		

Fig. 1. Example of the daily medical report from the World Games 2017

Table 3. Distribution of medical condition categories among patient medical interventions and hospital transports by group and tournament

Medical condition	Athletes				Non-athletes			
	medical intervention (%)		hospital transport (%)		medical intervention (%)		hospital transport (%)	
	WG 2017	EG 2023	WG 2017	EG 2023	WG 2017	EG 2023	WG 2017	EG 2023
Injury-related conditions								
Severe injury	42 (18.4)	30 (14.0)	27 (75.0)	24 (57.1)	14 (4.7)	9 (3.2)	7 (43.8)	8 (40.0)
Mild injury	69 (30.3)	58 (27.1)	7 (19.4)	15 (35.7)	76 (25.5)	42 (14.9)	4 (25.0)	4 (20.0)
Total injury	111 (48.7)	88 (41.1)	34 (94.4)	39 (92.9)	90 (30.2)	51 (18.1)	11 (68.8)	12 (60.0)
Illness-related conditions								
Severe illness	20 (8.8)	34 (15.9)	2 (5.6)	3 (7.1)	69 (23.2)	36 (12.8)	5 (31.3)	7 (35.0)
Mild illness	97 (42.5)	92 (43.0)	0 (0)	0 (0.0)	139 (46.6)	194 (69.0)	0 (0)	1 (5.0)
Total illness	117 (51.3)	126 (58.9)	2 (5.6)	3 (7.1)	208 (69.8)	230 (81.9)	5 (31.3)	8 (40.0)
Total medical conditions	228 (100)	214 (100)	36 (100)	42 (100)	298 (100)	281 (100)	16 (100)	20 (100)

WG 2017 – World Games 2017; EG 2023 – European Games 2023.

including pre-tournament days. The tournaments were not associated with any mass-casualty incidents, major public health threats, or fatalities.

Medical interventions and conditions in athletes

The athlete group reported 442 medical interventions and 78 hospital transports (46,127 participant-days). The pooled incidence rates for medical interventions and hospital transports were 9.58 (95% CI: 8.73–10.50) and 1.69 (95% CI: 1.36–2.09) per 1,000 participant-days, respectively. Injuries were related to 199 medical interventions and 73 hospital transports. The pooled injury incidence rate was 5.90 (95% CI: 5.24–6.62). The incidence rates for injury-related medical interventions and hospital transports were 4.31 (95% CI: 3.76–4.93) and 1.58 (95% CI: 1.26–1.97), respectively. Illnesses required 243 medical interventions and 5 hospital transports. The pooled illness incidence rate was 5.38 (95% CI: 4.75–6.07). The incidence rates for illness-related medical interventions and hospital transports were 5.27 (95% CI: 4.65–5.95) and 0.11 (95% CI: 0.05–0.22), respectively. Mild medical conditions accounted for 338 medical interventions and hospital transports, with a pooled incidence rate of 7.33 (95% CI: 6.59–8.13), whereas severe medical conditions accounted for 182 medical interventions and hospital transports, with an incidence rate of 3.95 (95% CI: 3.41–4.54).

Data on total medical interventions and medical conditions for WG 2017, EG 2023, and the pooled cohort are presented in Tables 4–6.

Medical interventions and conditions in non-athletes

The non-athlete group reported 579 medical interventions and 36 hospital transports (355,215 participant-days). The pooled incidence rates for medical interventions and

hospital transports were 1.63 (95% CI: 1.50–1.77) and 0.10 (95% CI: 0.07–0.14), respectively. Injuries were related to 141 medical interventions and 23 hospital transports. The pooled injury incidence rate was 0.46 (95% CI: 0.40–0.53). The incidence rates for injury-related medical interventions and hospital transports were 0.40 (95% CI: 0.34–0.47) and 0.06 (95% CI: 0.04–0.09), respectively. Illnesses required 438 medical interventions and 13 hospital transports. The pooled illness incidence rate was 1.27 (95% CI: 1.16–1.39). The incidence rates for illness-related medical interventions and hospital transports were 1.23 (95% CI: 1.12–1.35) and 0.04 (95% CI: 0.02–0.06), respectively. Mild medical conditions accounted for 460 medical interventions and hospital transports, with a pooled incidence rate of 1.29 (95% CI: 1.18–1.42), whereas severe medical conditions accounted for 155, with an incidence rate of 0.44 (95% CI: 0.37–0.51).

Athlete and non-athlete groups comparison

Compared with non-athletes, athletes demonstrated consistently higher incidence rates across all analyzed outcomes. In the pooled cohort, the IRR for total medical interventions was 5.88 (95% CI: 5.19–6.65), for injury-related medical interventions 10.87 (95% CI: 8.76–13.49), and for illness-related medical interventions 4.27 (95% CI: 3.65–5.00). Hospital transport rates were substantially higher among athletes. The pooled IRR for total hospital transports was 16.69 (95% CI: 11.24–24.77), 24.44 for injury-related hospital transports (95% CI: 15.30–39.05), and 2.96 for illness-related hospital transports (95% CI: 1.06–8.31). The latter estimate should be interpreted cautiously because of sparse event counts. Athletes also had a higher incidence of both mild and severe medical conditions, with pooled IRRs of 5.66 (95% CI: 4.92–6.51) and 9.04 (95% CI: 7.30–11.20), respectively. The observed differences were not limited to overall event rates but also reflected distinct

Table 4. Incidence rate per 1,000 participant-days (95% CI) and number per 100 persons (95% CI) for total medical interventions and hospital transports, for athletes and non-athletes, by the WG 2017, the EG 2023, and pooled cohort

Population and outcome	WG 2017	EG 2023	Pooled cohort
Total			
Population	173,922	190,965	364,887
Participant-days*	183,417	217,925	401,342
Medical interventions	526	495	1,021
Incidence rate (95% CI)	2.87 (2.63–3.11)	2.27 (2.08–2.47)	2.54 (2.39–2.70)
Number per 100 persons (95% CI)	0.30 (0.28–0.33)	0.26 (0.24–0.28)	0.28 (0.26–0.30)
Hospital transports	52	62	114
Incidence rate (95% CI, PA)	0.28 (0.21–0.36)	0.28 (0.22–0.36)	0.28 (0.24–0.34)
Number per 100 persons (95% CI, PA)	0.03 (0.02–0.04)	0.03 (0.02–0.04)	0.03 (0.03–0.04)
Mild medical conditions	392	406	798
Incidence rate (95% CI)	2.14 (1.93–2.35)	1.86 (1.69–2.05)	1.98 (1.86–2.13)
Number per 100 persons (95% CI)	0.23 (0.20–0.25)	0.21 (0.19–0.23)	0.22 (0.20–0.23)
Severe medical conditions	186	151	337
Incidence rate (95% CI)	1.01 (0.87–1.16)	0.69 (0.59–0.81)	0.84 (0.75–0.93)
Number per 100 persons (95% CI)	0.11 (0.09–0.12)	0.08 (0.07–0.09)	0.09 (0.08–0.10)
Athletes			
Population	3,292	6,380	9,672
Participant-days*	12,787	33,340	46,127
Medical interventions	228	214	442
Incidence rate (95% CI)	17.83 (15.66–20.22)	6.42 (5.62–7.31)	9.58 (8.73–10.50)
Number per 100 persons (95% CI)	6.93 (6.10–7.85)	3.35 (2.94–3.82)	4.57 (4.17–5.00)
Hospital transports	36	42	78
Incidence rate (95% CI)	2.82 (2.04–3.81)	1.26 (0.93–1.67)	1.69 (1.36–2.09)
Number per 100 persons (95% CI)	1.09 (0.79–1.51)	0.66 (0.49–0.89)	0.81 (0.65–1.00)
Mild medical conditions	173	165	338
Incidence rate (95% CI)	13.53 (11.66–15.62)	4.95 (4.25–5.73)	7.33 (6.59–8.13)
Number per 100 persons (95% CI)	5.26 (4.55–6.07)	2.59 (2.22–3.00)	3.49 (3.14–3.88)
Severe medical conditions	91	91	182
Incidence rate (95% CI)	7.12 (5.80–8.65)	2.73 (2.22–3.32)	3.95 (3.41–4.54)
Number per 100 persons (95% CI)	2.76 (2.23–3.38)	1.43 (1.16–1.75)	1.88 (1.63–2.17)
Non-athletes			
Population	170,630	184,585	355,215
Participant-days*	170,630	184,585	355,215
Medical interventions	298	281	579
Incidence rate (95% CI)	1.75 (1.56–1.95)	1.52 (1.35–1.71)	1.63 (1.50–1.77)
Number per 100 persons (%) (95% CI)	0.17 (0.16–0.20)	0.15 (0.14–0.17)	0.16 (0.15–0.18)
Hospital transports	16	20	36
Incidence rate (95% CI)	0.09 (0.06–0.14)	0.11 (0.07–0.16)	0.10 (0.07–0.14)
Number per 100 persons (%) (95% CI)	0.009 (0.006–0.015)	0.011 (0.007–0.017)	0.01 (0.007–0.014)
Mild medical conditions	219	241	460
Incidence rate (95% CI)	1.28 (1.12–1.46)	1.31 (1.15–1.48)	1.29 (1.18–1.42)
Number per 100 persons (95% CI)	0.13 (0.11–0.15)	0.13 (0.11–0.15)	0.13 (0.12–0.14)
Severe medical conditions	95	60	155
Incidence rate (95% CI)	0.56 (0.46–0.67)	0.33 (0.25–0.41)	0.44 (0.37–0.51)
Number per 100 persons (95% CI)	0.06 (0.05–0.07)	0.03 (0.03–0.04)	0.04 (0.04–0.05)

*Participant-days were calculated as the total number of days of exposure contributed by all participants. WG 2017 – World Games 2017; EG 2023 – European Games 2023; PA – Poisson approximation; 95% CI – 95% confidence interval.

Table 5. Incidence rate per 1,000 participant-days (95% CI) and number per 100 persons (95% CI) for injury-related medical interventions and hospital transports, athletes and non-athletes, by the WG 2017, the EG 2023, and pooled cohort

Population and outcome	WG 2017	EG 2023	Pooled cohort
Total			
Population	173,922	190,965	364,887
Participant-days*	183,417	217,925	401,342
Total injuries	246	190	436
Incidence rate (95% CI)	1.34 (1.18–1.51)	0.87 (0.75–1.00)	1.09 (0.99–1.19)
Number per 100 persons (95% CI)	0.14 (0.12–0.16)	0.10 (0.09–0.11)	0.12 (0.11–0.13)
Injury-related medical interventions	201	139	340
Incidence rate (95% CI)	1.10 (0.95–1.25)	0.64 (0.54–0.75)	0.85 (0.76–0.94)
Number per 100 persons (95% CI)	0.12 (0.10–0.13)	0.07 (0.06–0.09)	0.09 (0.08–0.10)
Injury-related hospital transports	45	51	96
Incidence rate (95% CI)	0.25 (0.18–0.32)	0.23 (0.18–0.30)	0.24 (0.20–0.29)
Number per 100 persons (95% CI)	0.03 (0.02–0.03)	0.03 (0.02–0.03)	0.03 (0.02–0.03)
Athletes			
Population	3,292	6,380	9,672
Participant-days*	12,787	33,340	46,127
Injury-related medical interventions	111	88	199
Incidence rate (95% CI)	8.68 (7.21–10.37)	2.64 (2.14–3.22)	4.31 (3.76–4.93)
Number per 100 persons (95% CI)	3.37 (2.81–4.04)	1.38 (1.12–1.70)	2.06 (1.79–2.36)
Injury-related hospital transports	34	39	73
Incidence rate (95% CI)	2.66 (1.91–3.62)	1.17 (0.86–1.56)	1.58 (1.26–1.97)
Number per 100 persons (95% CI)	1.03 (0.74–1.44)	0.61 (0.45–0.83)	0.75 (0.60–0.95)
Non-athletes			
Population	170,630	184,585	355,215
Participant-days*	170,630	184,585	355,215
Injury-related medical interventions	90	51	141
Incidence rate (95% CI)	0.53 (0.43–0.64)	0.28 (0.21–0.36)	0.40 (0.34–0.47)
Number per 100 persons (95% CI)	0.05 (0.04–0.07)	0.03 (0.02–0.04)	0.04 (0.03–0.05)
Injury-related hospital transports	11	12	23
Incidence rate (95% CI)	0.06 (0.04–0.11)	0.07 (0.04–0.10)	0.06 (0.04–0.09)
Number per 100 persons (95% CI)	0.006 (0.004–0.012)	0.0065 (0.0037–0.0114)	0.006 (0.004–0.010)

*Participant-days were calculated as the total number of days of exposure contributed by all participants. WG 2017 – World Games 2017; EG 2023 – European Games 2023; 95% CI – 95% confidence interval.

patterns of medical conditions. Injury-related events accounted for a larger proportion of medical interventions among athletes, whereas illness-related events represented a relatively greater proportion among non-athletes.

Across tournaments, the total population demonstrated higher overall medical intervention rates during WG 2017 compared with EG 2023 (IRR = 1.26, 95% CI: 1.12–1.43), injury-related medical interventions (IRR = 1.72, 95% CI: 1.38–2.13), and severe medical conditions (IRR = 1.46, 95% CI: 1.18–1.81), whereas hospital transport rates remained comparable between tournaments. Among athletes, most medical outcomes occurred at significantly higher rates during WG 2017 than during EG 2023. In contrast, among non-athletes, most between-tournament differences were small and not statistically significant, except for severe medical conditions (IRR = 1.71, 95% CI:

1.24–2.37) and injury-related medical interventions (IRR = 1.91, 95% CI: 1.35–2.69).

Table 7 summarizes the comparative analyses of WG 2017, EG 2023, and the pooled cohort. Tables 4–6 present descriptive incidence estimates with 95% CIs.

Sport discipline-specific hospital transports and related medical conditions

Among the disciplines, the highest incidence of hospital transports was observed in muaythai – 28.41 (95% CI: 9.22–66.30) and American football – 27.78 (95% CI: 13.32–51.08) at WG 2017; BMX freestyle cycling – 21.28 (95% CI: 2.58–76.86) at EG 2023; sumo – 15.79 (95% CI: 3.26–46.14) and beach handball – 13.47 (95% CI: 6.16–25.58) at WG 2017; taekwondo – 6.05 (95% CI: 2.22–13.16) and rugby

Table 6. Incidence rate per 1,000 participant-days (95% CI) and number per 100 persons (95% CI) for illness-related medical interventions and hospital transports, athletes and non-athletes, by the WGs 2017, the EG 2023, and pooled cohort

Population and outcome	WG 2017	EG 2023	Pooled cohort
Total			
Population	173,922	190,965	364,887
Participant-days*	183,417	217,925	401,342
Total illnesses	332	367	699
Incidence rate (95% CI)	1.81(1.63–2.01)	1.68 (1.52–1.86)	1.74 (1.62–1.87)
Number per 100 persons (95% CI)	0.19 (0.17–0.21)	0.19 (0.17–0.21)	0.19 (0.18–0.21)
Illness-related medical interventions	325	356	681
Incidence rate (95% CI)	1.77 (1.59–1.97)	1.63 (1.47–1.80)	1.70 (1.57–1.83)
Number per 100 persons (95% CI)	0.18 (0.17–0.21)	0.19 (0.16–0.20)	0.19 (0.17–0.20)
Illness-related hospital transports	7	11	18
Incidence rate (95% CI, PA)	0.04 (0.02–0.07)	0.05 (0.03–0.08)	0.04 (0.03–0.07)
Number per 100 persons (95% CI, PA)	0.004 (0.002–0.008)	0.006 (0.003–0.010)	0.005 (0.003–0.008)
Athletes			
Population	3,292	6,380	9,672
Participant-days*	12,787	33,340	46,127
Illness-related medical interventions	117	126	243
Incidence rate (95% CI)	9.15 (7.64–10.88)	3.78 (3.17–4.46)	5.27 (4.65–5.95)
Number per 100 persons (95% CI)	3.55 (2.97–4.24)	1.97 (1.64–2.34)	2.51 (2.22–2.84)
Illness-related hospital transports	2	3	5
Incidence rate (95% CI, PA)	0.16 (0.05–0.43)	0.09 (0.03–0.22)	0.11 (0.05–0.22)
Number per 100 persons (95% CI, PA)	0.061 (0.017–0.221)	0.05 (0.016–0.14)	0.05 (0.02–0.12)
Non-athletes			
Population	170,630	184,585	355,215
Participant-days*	170,630	184,585	355,215
Illness-related medical interventions	208	230	438
Incidence rate (95% CI)	1.22 (1.06–1.39)	1.25 (1.09–1.41)	1.23 (1.12–1.35)
Number per 100 persons (95% CI)	0.12 (0.11–0.14)	0.12 (0.10–0.14)	0.12 (0.11–0.14)
Illness-related hospital transports	5	8	13
Incidence rate (95% CI, PA)	0.03 (0.01–0.06)	0.04 (0.02–0.07)	0.04 (0.02–0.06)
Number per 100 persons (95% CI, PA)	0.003 (0.001–0.007)	0.004 (0.001–0.008)	0.004 (0.002–0.006)

*Participant-days were calculated as the total number of days of exposure contributed by all participants. WG 2017 – World Games 2017; EG 2023 – European Games 2023; PA – Poisson approximation; 95% CI – 95% confidence interval.

Table 7. Comparative analysis of medical event rates in athletes and non-athletes during the WG 2017, the EG 2023, and between tournaments using Poisson IRRs and 95% CIs

Outcome	Athletes vs non-athletes (pooled cohort) IRR (95% CI)	WG 2017 vs EG 2023 (total population) IRR (95% CI)	WG 2017 vs EG 2023 IRR among athletes (95% CI)	WG 2017 vs EG 2023 IRR among non-athletes (95% CI)
Total medical interventions	5.88 (5.19–6.65)	1.26 (1.12–1.43)	2.78 (2.31–3.35)	1.15 (0.97–1.35)
Total hospital transports	16.69 (11.24–24.77)	1.00 (0.69–1.44)	2.23 (1.43–3.49)	0.87 (0.45–1.67)
Mild medical conditions	5.66 (4.92–6.51)	1.15 (1.00–1.32)	2.73 (2.21–3.38)	0.98 (0.82–1.18)
Severe medical conditions	9.04 (7.30–11.20)	1.46 (1.18–1.81)	2.61 (1.95–3.49)	1.71 (1.24–2.37)
Injury-related medical interventions	10.87 (8.76–13.49)	1.72 (1.38–2.13)	3.29 (2.49–4.35)	1.91 (1.35–2.69)
Injury-related hospital transports	24.44 (15.30–39.05)	1.05 (0.70–1.57)	2.27 (1.44–3.60)	0.99 (0.44–2.25)
Illness-related medical interventions	4.27 (3.65–5.00)	1.08 (0.93–1.26)	2.42 (1.88–3.11)	0.98 (0.81–1.18)
Illness-related hospital transports*	2.96 (1.06–8.31)	0.76 (0.29–1.95)	1.74 (0.29–10.40)	0.68 (0.22–2.07)

*Interpret with caution because of sparse event counts. IRR – incidence rate ratio; 95% CI – 95% confidence interval; WG 2017 – World Games 2017; EG 2023 – European Games 2023.

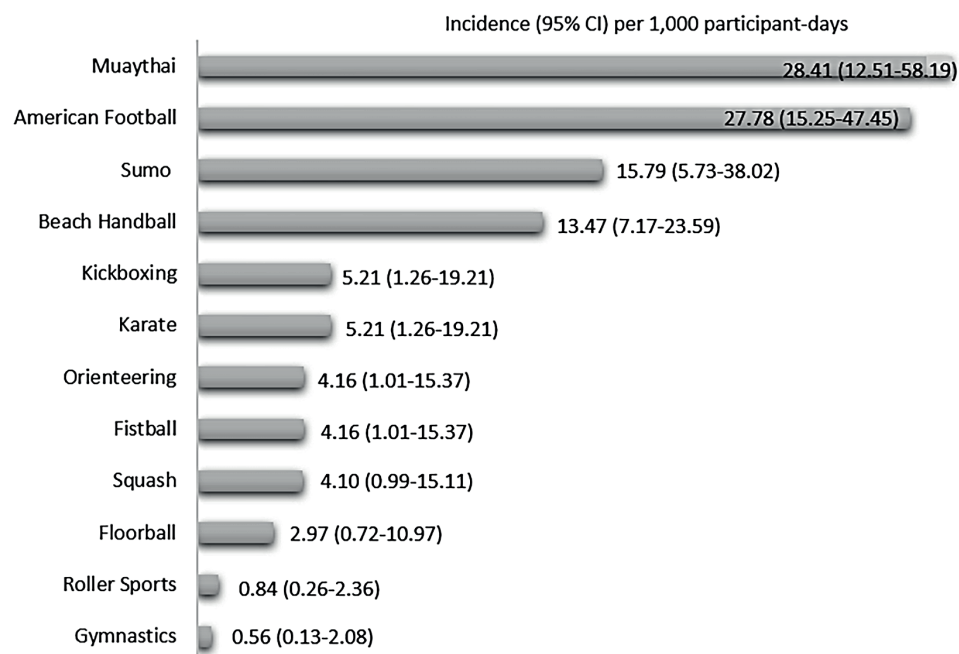


Fig. 2. Hospital transport incidence rate in athlete group by disciplines during the World Games 2017

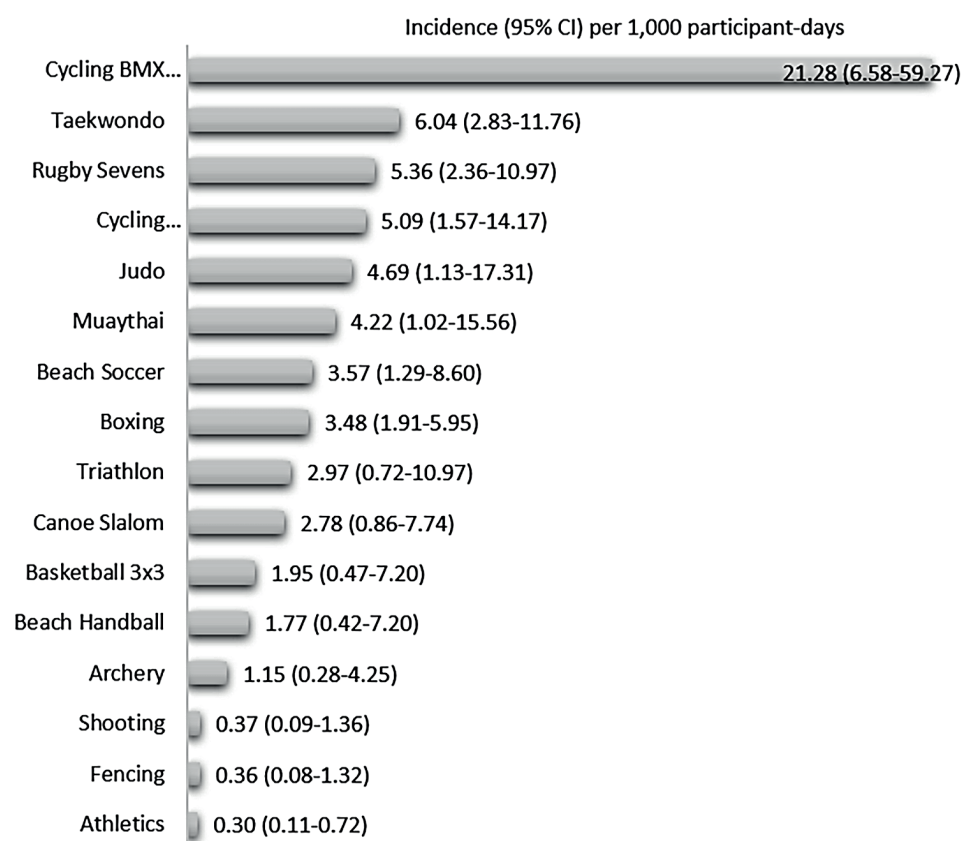


Fig. 3. Hospital transport incidence rate in athlete group by sport disciplines during the European Games 2023

sevens 5.36 (95% CI: 1.74–12.51) at EG 2023. Figures 2,3 present tournament data. These disciplines accounted for 40 of 78 hospital transports (51.3%). In the pooled athlete cohort, hospital transports were predominantly injury-related (93.6%). The highest IRs were reported for upper limb (0.50, 95% CI: 0.32–0.74), head (IR = 0.41, 95% CI: 0.25–0.64), and lower limb injuries (IR = 0.39, 95% CI: 0.23–0.62). Figures 4,5 present tournament data.

Discussion

This study analyzed patterns of medical interventions and conditions managed by the VMTs in athlete and non-athlete populations during 2 major international multi-sport tournaments held in Poland – the WG 2017 and the EG 2023. The primary outcomes were incidence rates of medical interventions and hospital transports;

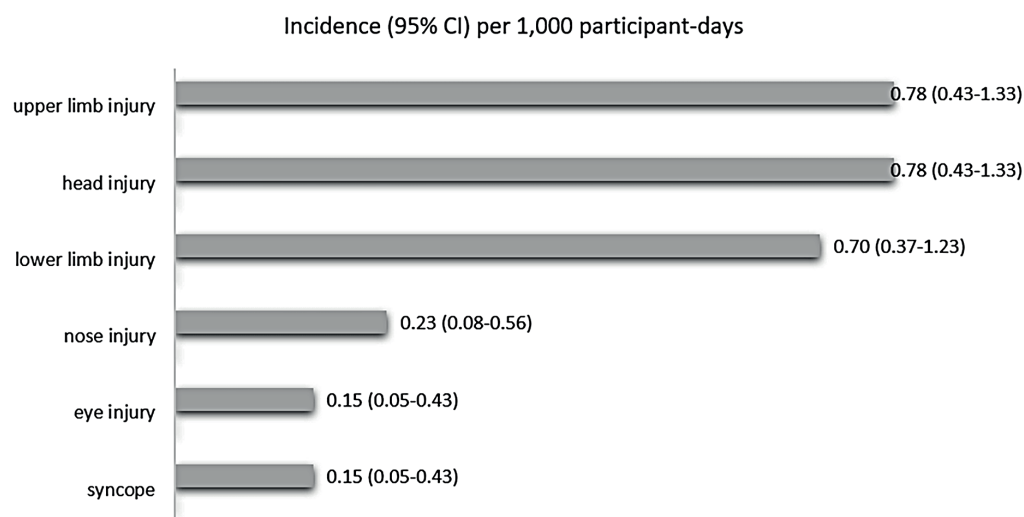


Fig. 4. Medical conditions related to hospital transport incidence rate in athlete group during the World Games 2017

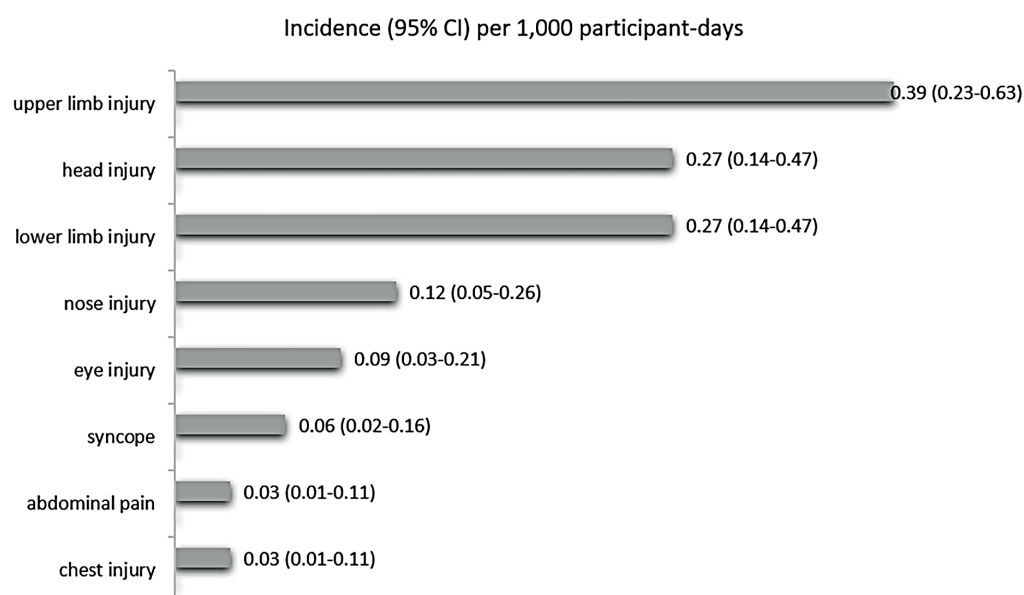


Fig. 5. Medical conditions related to hospital transport incidence rate in the athlete group during the European Games 2023

secondary analyses examined clinical characteristics and the severity of medical conditions to determine patterns of medical demand across participant groups.

Patterns of medical demands

Overall, both tournaments demonstrated a low incidence of medical interventions, ranging from 2.27 to 2.87 per 1,000 participant-days, with the hospital transport incidence approx. nine-to-tenfold lower (0.28 per 1,000 participant-days). Mild medical conditions predominated in all analyzed groups. Among participants requiring hospital transports, injury-related conditions predominated, with incidence rates fivefold higher than those for illnesses. These findings underscored the important role of VMTs in managing most medical interventions on-site, limiting the need for hospital transport and EMS utilization. Hospital transports were primarily required for severe and injury-related conditions. This pattern is further supported by the predominance of illnesses over injuries (IR 1.74

vs 1.09 per 1,000 participant-days) and mild over severe medical conditions (IR 1.99 vs 0.84). However, these results should be interpreted with caution, given the estimation approach used for the spectator group and the reliance on VMTs as the sole data source for athletes.

The athletes

In the athlete group, 5.4% of individuals required medical interventions or hospital transports from VMTs. The overall pattern of medical events in this group differed from that observed in the total cohort. Hospital transports occurred over fivefold less frequently than on-site medical interventions. Mild medical conditions continued to predominate by approximately twofold. Notably, injury and illness incidences were comparable. As expected, injury-related conditions predominated among hospital transports in this group and across all recorded hospital transports.

The incidence of injuries in the analyzed tournaments (IR = 4.31, 95% CI: 3.76–4.93) was lower than

the benchmarks reported for the summer OGs: 6.01 (95% CI: 5.65–6.38) in Beijing 2008,^{12,13} 7.58 (95% CI: 7.18–7.99) in London 2012,^{12,14} 5.74 (95% CI: 5.41–6.09) in Rio de Janeiro 2016,^{12,15} and 5.38 (95% CI: 5.06–5.72) in Tokyo 2020.^{12,16} The summer Youth Olympic Games (YOG) in Buenos Aires 2018 demonstrated an even higher injury incidence of 11.95 (95% CI: 11.03–12.93).^{12,17} The results observed in this study were closer to those reported for the 2021 Summer World University Games (WUG), which involved 6,500 athletes and reported an injury incidence of 4.9 (95% CI: 4.3–5.4).¹⁸

The observed illness incidence (IR = 5.27, 95% CI: 4.65–5.95) was higher than that reported for the OG: 4.22 (95% CI: 3.92–4.53) in 2012,^{12,14} 3.20 (95% CI: 2.95–3.46) in 2016,^{12,15} and 2.28 (95% CI: 2.07–2.50) in 2020,^{12,16} as well as for WUG 2021 (2.5, 95% CI: 2.1–2.9).¹⁸ YOG 2018 demonstrated a higher illness incidence of 6.45 (95% CI: 5.78–7.18).¹⁷ These differences may reflect variations in event scale, competitive demands, and reporting systems. During the OG, medical reporting relied primarily on team physicians, whereas in other MSTs, reporting relied on data collected by VMTs.

The lower incidence of venue medical interventions observed during EG 2023 may also reflect differences in medical service organization. At WG 2017, VMTs handled most athlete medical care, whereas at EG 2023, team physicians managed a substantial part of the medical workload. This difference in data-collection procedures may have led to underreporting of mild medical conditions.

The non-athletes

The profile of medical interventions in the non-athlete group differed from that observed in athletes. Only 0.16% of non-athletes required medical interventions at the venues and 0.01% required hospital transports. Injury-related medical intervention rates were lower, whereas illness-related rates were comparable to those observed in the total cohort. Hospital transport rates were approximately fourfold lower for injuries and comparable for illnesses, although illness-related estimates need to be interpreted with caution due to sparse event counts. Mild medical conditions were similar, while severe conditions were twofold less frequent. The patterns were consistent across both tournaments and aligned with those reported at previous events, including the Melbourne 2006 Commonwealth Games¹⁶ and the 2023 Ryder Cup.¹⁷

Difference in medical patterns

Comparisons between groups revealed distinct patterns of medical demands. Athletes had consistently higher rates of both on-site medical interventions and hospital transports than non-athletes across all analyzed outcomes, with the largest between-group differences for injury-related medical interventions, injury-related hospital transports,

and severe medical conditions. Although illness-related medical interventions were also more frequent among athletes, illness-related hospital transports remained very infrequent in both groups. The distribution of medical conditions also differed between groups, with injury-related events representing a greater proportion of medical interventions among athletes, whereas non-athletes were characterized predominantly by illness-related and mild medical conditions. Furthermore, although mild medical conditions predominated in both groups, the relative difference between athletes and non-athletes was more pronounced for severe than for mild conditions. Collectively, these findings indicate that medical interventions in athletes were characterized by a substantially greater burden of injury-related and severe medical conditions, whereas illness-related and mild medical conditions predominated among non-athletes.

This overall pattern is consistent with the observed trends in other major international sporting tournaments.^{12–24,28–32} Figure 6 presents the comparative patterns of medical interventions between athlete and non-athlete groups.

Determinants in hospital transport in athletes

In the present analysis, sport disciplines associated with a higher frequency of hospital transports were identified. Muaythai, American football, BMX freestyle cycling, sumo, and beach handball, each with hospital transport incidences exceeding 10 per 1,000 participant-days, represent contact, high-speed, or team disciplines with an inherently elevated risk of trauma. While several of these disciplines, such as taekwondo, football, handball, rugby, and cycling, align with Olympic disciplines known for elevated injury risk,^{12–17} a number of non-Olympic disciplines represented in these tournaments also showed increased hospital transport rates. Boxing, despite accounting for the highest absolute number of hospital transports during EG 2023, exhibited a comparatively lower incidence. Among medical conditions requiring hospital transport, limb and head injuries predominated, consistent with previous reports.¹² However, in the analyzed cohort, upper limb injuries predominated over lower limb injuries. Interpreting discipline-specific hospital transport rates requires caution, as several disciplines had few events, resulting in wide CIs and limited precision. Consequently, the observed differences may primarily reflect operational patterns relevant to medical resource planning during multisport tournaments rather than definitive evidence of true discipline-specific risk.

Practical implications

This study provides data-driven insights into medical demands during MSTs. The inclusion of all participant groups enabled a comprehensive assessment of medical

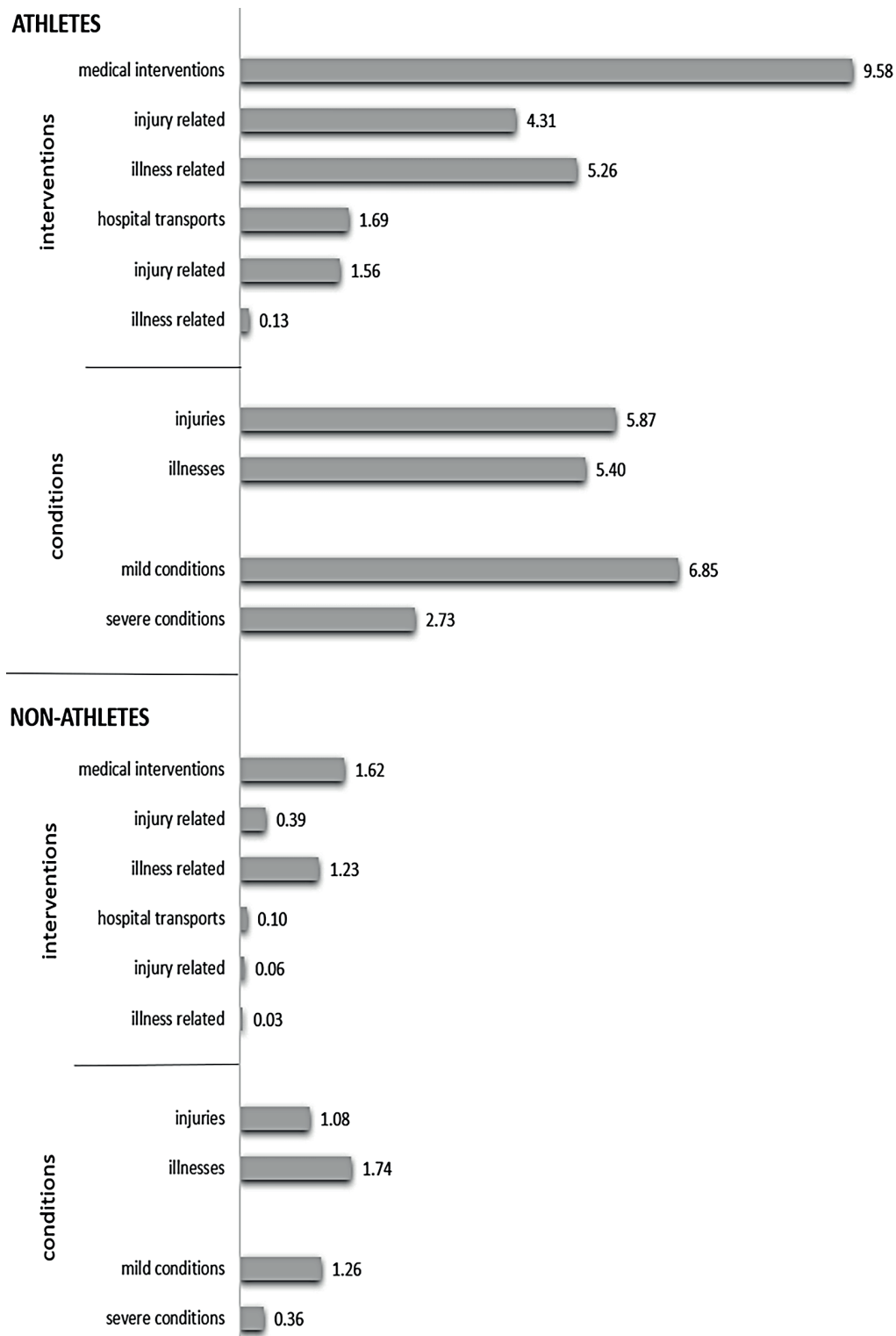


Fig. 6. The comparative patterns of medical demands between pooled athlete and non-athlete groups

needs at the venues.^{26,28–35} Distinct patterns of medical interventions and conditions between groups provide a basis for targeted prevention strategies and more informed medical planning for future tournaments.

The predominance of mild medical conditions and on-site interventions across all groups supports the overall medical safety of these MSTs.^{30–36} The higher incidence of illnesses in both groups should be considered in planning for future events. The overall low number of hospital

transports guides EMS involvement and referral hospital selection, while underscoring the need for continuous coordination between entities.^{2–6,31–37} The profile of hospital transports may inform the selection of referral hospitals,^{33,36,37} while the predominance of limb and head injuries and the identification of higher-risk sport disciplines support tailored medical planning.

As tournaments continue to grow in scale and complexity, continuous surveillance of medical data is essential

to ensure the provision of required medical services, to monitor trends in medical needs, and to assess the effectiveness of the prevention strategies implemented.^{12,34–38} A structured bottom-up reporting system enables reliable data collection for subsequent analyses. Integration of information from other stakeholders (team physicians, team managers, and event organizers) is required for a more comprehensive assessment of medical demands. Inclusion of the referral hospital data, particularly regarding patient follow-up, would strengthen the evidence for medical analyses.^{28,32,39} For non-athletes, accurate attendance data and demographic profiling would further enhance understanding of medical needs.^{2–10,25,26,31–33}

Expanding reporting systems in alignment with IOC surveillance frameworks, while adapting them to include non-athlete populations, would improve comparability and analytical potential across MSTs. Such standardization would ultimately strengthen medical data consistency.¹²

Limitations of the study

First, the non-athlete population was estimated from ticketing data, which may have overestimated attendance and, consequently, underestimated medical service utilization rates among spectators. Second, athlete-related data did not include reports from team physicians, potentially underrepresenting minor or sport-specific medical conditions managed outside VMTs. Importantly, the on-site medical response captured all conditions requiring hospital transport and included them in the dataset. In addition, the low number of hospital transports, particularly for illness-related conditions, limited the precision of incidence estimates. The dataset did not allow more detailed analyses, such as gender differences. Furthermore, the analysis included complete tournament populations rather than randomly selected samples. Therefore, the findings should be generalized with caution, and the reported CIs and statistical comparisons should be interpreted primarily as measures of variability and tools for comparison between the analyzed tournaments and participant groups. Finally, both tournaments were held in summer conditions, which should be considered when comparing these findings with events conducted in different environmental contexts.

Conclusions

This prospective study demonstrated distinct patterns in the incidence, type, and severity of medical interventions managed by VMTs among athlete and non-athlete populations during 2 international multi-sport tournaments. Athletes experienced substantially higher incidences of medical interventions and hospital transports across all analyzed outcomes, with the greatest between-group differences observed for injury-related and severe

medical conditions. Although mild conditions predominated in both groups, illness-related conditions represented a relatively greater proportion of medical interventions among non-athletes, whereas injury-related conditions represented a greater proportion among athletes. Hospital transports were relatively uncommon in both groups but occurred substantially more frequently among athletes, with the vast majority being injury-related.

Comparisons between tournaments indicated generally similar patterns of medical events, although athletes demonstrated higher incidences of most medical outcomes during WG 2017 than during EG 2023. The combined analysis of athlete and non-athlete populations provided a comprehensive assessment of the overall medical workload encountered by VMTs and highlighted the value of standardized surveillance for comparing healthcare demands across participant groups and tournaments. Within the athlete cohort, several sport disciplines, including muaythai, American football, BMX freestyle cycling, sumo, and beach handball, showed higher hospital transport rates; however, the absolute number of events was low. Upper limb, head, and lower limb injuries were the most frequent conditions requiring hospital transport. These findings support evidence-based medical planning by accounting for differences in medical interventions, hospital transport requirements, and medical condition profiles between participant groups and sport disciplines at future international multi-sport tournaments.

Data Availability Statement

The aggregated dataset supporting the findings of this study is available in Zenodo at <https://doi.org/10.5281/zenodo.21886930>. No individual-level or personally identifiable data are included.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Generative AI (Google Gemini 2.5 Pro, Google DeepMind) was used to improve language and readability; the authors reviewed and take full responsibility for the content.

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Clinical aspects of inpatient suicides: A retrospective analysis of suicides from 2008 to 2019 in 9 hospitals in North Rhine-Westphalia

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Abstract

Background. Suicides represent a serious public health problem worldwide. An increase in suicide rates was observed among psychiatric inpatients.

Objectives. The aim of this study was to examine differences in inpatient suicides across age groups and to derive clinical implications from special features of inpatient suicide.

Materials and methods. A total of 229 (partially) inpatient suicides were retrospectively analyzed based on standardized report protocols. Data included information on sociodemographic background, medication, diagnoses, duration of hospitalization, suicide method, location, weekday, and potential stressors. Suicide rates per admission were calculated by sex and age group. Age-related differences in categorical variables were analyzed using χ^2 tests.

Results. From 2008 to 2019, the overall suicide rate ranged from 0.06% to 0.03% suicides/admission. Suicide rates per admission, stratified by age group, showed a significant increase for both women and men aged 60–69 years. Age stratification (χ^2 tests including post hoc tests with Bonferroni correction) showed differences in diagnoses, stressors, and medication. Hanging was the most common suicide method, followed by railway suicide. More than half of the suicides occurred outside the hospital environment, during hospital leave approved by staff.

Conclusions. The suicide rate per duration of hospitalization increased after the first month of treatment, which may highlight the severity of the underlying mental illness, resulting in a longer hospitalization period and potential therapy resistance, so approved periods of leave should be considered carefully. Awareness of somatic comorbidities and chronic pain during the therapeutic process is important, especially for older patients. Hanging is the most common method of suicide, whereas railway suicide is the most common method in younger age groups. These findings emphasize the importance of preventive construction measures.

Key words: suicide (attempted), inpatients, mental disorders, age factors, risk factors

Highlights

- Inpatient suicide rates increased significantly among women and men aged 60–69 years, highlighting older psychiatric inpatients as a high-risk group.
- Suicide risk rose after the 1st month of hospitalization, suggesting an association with illness severity, prolonged treatment, and possible therapy resistance.
- Hanging was the most frequent suicide method, while suicide by railway was more common in younger patients, underscoring the need for targeted preventive and structural safety measures.
- More than half of inpatient suicides occurred during approved hospital leave, with nearly half taking place at week-ends. This emphasizes the importance of careful leave decisions and enhanced risk assessment, particularly for patients with somatic comorbidities and chronic pain.

Background

Suicide remains a substantial global issue. Only a minority of suicides occur while the person concerned is under treatment in a psychiatric hospital. However, these events are particularly tragic, since help was close. Ambivalence toward treatment and care might result in a decision to disengage from medical care and commit suicide. In 2019, the World Health Organization (WHO) reported more than 700,000 suicides worldwide.¹ The estimates for suicide attempts are up to 30 times higher than those for completed suicides.² In 2019, the suicide rate was 8.27 per 100,000 people in the general population in Germany.¹ While there had been a constant decrease in suicide rates over the past decades in Germany, this trend was reversed in 2022, as the suicide rate increased to 12.1 per 100,000 people in the general population.³ The estimated suicide rate among psychiatric inpatients worldwide over the last 60 years was 147 per 100,000 admissions, which is higher than the suicide rate in the general population.⁴ Older studies in Germany reported 1,687⁵ and 1,312 admissions per suicide.⁶ More recent studies reported 1,389 admissions per suicide^{7,8} and 444 admissions per suicide.⁹

Although inpatient treatment in psychiatric departments is focused on the prevention of self-harm and suicidal behavior, some inpatients die by suicide during treatment. The risk of suicide is highest in the 1st week of admission and in the 1st week following discharge.^{10,11} The suicide rate after discharge was 100 times higher than the global rate in the general population and 3 times higher than the rate among psychiatric inpatients.¹⁰ Analysis of suicide risk in relation to psychiatric hospitalization showed that suicide risk was higher in patients receiving treatment for a shorter period.¹¹ Patients who did not complete treatment were at higher risk of repeated suicidal acts.¹² Furthermore, patients with suicidal ideation appeared to be more ambivalent toward psychotherapy and did not believe in the success of treatment.¹³

Busch et al. retrospectively analyzed suicidal ideation or behavior in patients who died by suicide during inpatient treatment. In this study, 78% denied suicidal ideation during the last therapeutic exchange.¹⁴ Even if psychiatric

treatment is specifically tailored to protect patients from suicide, in a small number of cases the fatal outcome cannot be prevented. Suicides can occur even in contexts of constant observation.¹⁵ Long-term risk factors for suicide include psychosis, major depressive disorder, and a history of previous suicide attempts, among others.¹⁶ Exploratory studies on inpatient suicide are necessary and important to improve inpatient management and minimize the risk of suicidal behavior.

Objectives

In this retrospective analysis of inpatient suicides, suicide rates for each year were calculated. Next, inpatient suicides were stratified by age, sex, diagnosis, suicide method, location of suicide, possible stressors, and medication. Due to the hospital setting, special features of inpatient treatment, such as the duration of treatment, type of leave, and day of the week, were also evaluated.

Materials and methods

Setting and subjects

Data on (partially) inpatient suicides were gathered from a group of 9 psychiatric hospitals in North Rhine-Westphalia, Germany. Four of these hospitals are located in predominantly urban catchment areas, while the other 5 are based in more rural communities. Altogether, across all 9 hospitals, approx. 60,000 cases are treated each year. The total number of annual inpatient admissions for all involved institutions was obtained from the municipal administration for each year (for a more detailed description, see Christl et al.¹⁷).

Eligibility criteria

Included were inpatient suicides on open as well as secured wards and in daycare units. Patients living in the department of rehabilitation and those who were younger

than 18 years were excluded. Suicides within 3 days after discharge were excluded due to documentation uncertainty.

Data and measures

In the hospitals concerned, suicide and other serious incidents must be reported to the hospital group headquarters using a standardized protocol. Immediately after the incident, the standardized protocol has to be generated by the physician and nurse in charge. Here, supervision of the treating physicians is established, and cases are critically re-evaluated with a supervising physician at the headquarters. The anonymous protocols were reviewed for the period from 2008 to 2019 to extract systematic information on patient suicides. The retrospective suicide cases were coded numerically. While most protocols were digitized, missing protocols were available on paper. Since only anonymous information had been recorded, data could easily be extracted in compliance with regulatory requirements.

The information to be obtained was limited to patient sociodemographic and clinical data. The information included sex, age, date of admission, the first 3 diagnoses coded according to the ICD-10 classification, weekday, duration of treatment, location of suicide, current medication, and potential stressors. The following information on location was obtained: on the ward, on hospital grounds, at home or nearby, at the parents' or partner's home, and outside the hospital or home environment. The medication was categorized into the following groups: antipsychotics, antidepressants, tranquilizers (benzodiazepines, low-potency antipsychotics such as promethazine, pipamperone, melperone, and chlorprothixene), Z-drugs, lithium, and other medication. The number of categorized drugs was counted. In addition, the leave policy was evaluated and categorized as follows: no approved leave, leave during the day, leave during the night, daycare, and unauthorized leave. The standardized protocols also included questions about potential triggers. These questions focused on what happened before and during the incident and what the consequences were. The treating and supervising physicians at the headquarters discussed possible stressors in retrospect. Possible stressors were then categorized into the following categories: family as well as partnership conflicts, physical complaints and pain, psychotic symptoms, financial problems, job-related problems, social problems, mental health (except for psychotic symptoms), death of someone close, and loss of autonomy.

Statistical analyses

Demographic and clinical variables were presented descriptively as means or percentages. The Kolmogorov–Smirnov test was performed to verify the normal distribution of age and duration of treatment (days). Differences in sex, diagnoses, suicide method, location of suicide,

weekday, medication, potential stressors, and duration of treatment stratified by age were evaluated with χ^2 tests for categorical variables. Missing data were not included. Post hoc tests were performed for all significant χ^2 tests. The expected subclass size was at least 5 in 75% of subclasses; otherwise, Fisher's exact test was used. The p-values were adjusted according to the Bonferroni correction (p_{corr}).

The statistical analysis was performed using IBM SPSS v. 28.0 (IBM Corp., Armonk, USA). The study was approved by the Ethics Committee of the Medical Faculty of Heinrich-Heine University (Düsseldorf, Germany; approval No. 4994).

Results

Suicide rates

From 2008 to 2019, a total of 246 reports of suicides were analyzed. In 12 of these cases, patients died by suicide within 3 days after discharge and were excluded from the investigation. Another 4 subjects who died by suicide were also excluded because they were living in a department of rehabilitation, which is a long-term care facility and cannot be compared with a psychiatric hospital setting. The youngest and oldest patients who died by suicide were both male, aged 15 and 88 years, respectively. Age was normally distributed (Supplementary Table 1; $p = 0.200$). The mean age was 50.9 years (standard deviation (SD): 16.3); 140 (61.1%) patients were male, while 89 (38.9%) were female. The 15-year-old patient was excluded from statistical analysis because he was the only minor. In total, 229 patients were included in the final analysis.

From 2008 to 2019, a total of 580,047 patients were treated across all hospitals under investigation. Based on the 229 registered suicides in this period, the overall suicide rate was 39.50 per 100,000 admissions (0.04%). The suicide rate per admission for men was higher than that for women in all years except for 2008. The overall suicide rate was 43.4 per 100,000 admissions (0.04%) for men and 34.5 per 100,000 admissions for women (0.03%; Fig. 1 and Supplementary Table 2). Among men, the total number of suicides was highest in 2013 ($n = 16$) and 2017 ($n = 17$). For women, the total number was highest in 2008 ($n = 12$) and 2017 ($n = 10$). Age stratification revealed that the suicide rate per admission significantly increased among men aged 60–69 and ≥ 70 years, and among women aged 60–69 years (Fig. 2).

Inpatient suicide stratified by age

Sex

In the sample studied, the sex ratio was 1.6:1 (140 men/89 women). In the period from 2008 to 2019, more men (54.3%) were admitted. While the sample did not differ

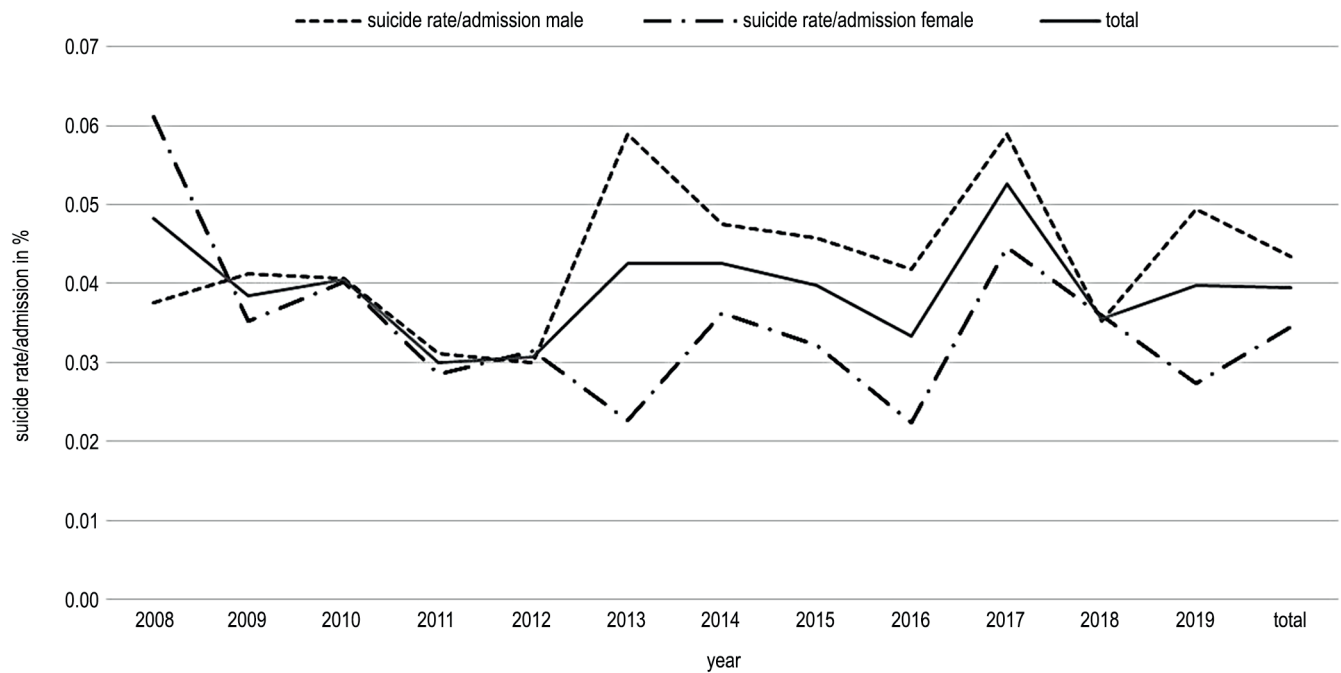


Fig. 1. Suicide rate/admission by year

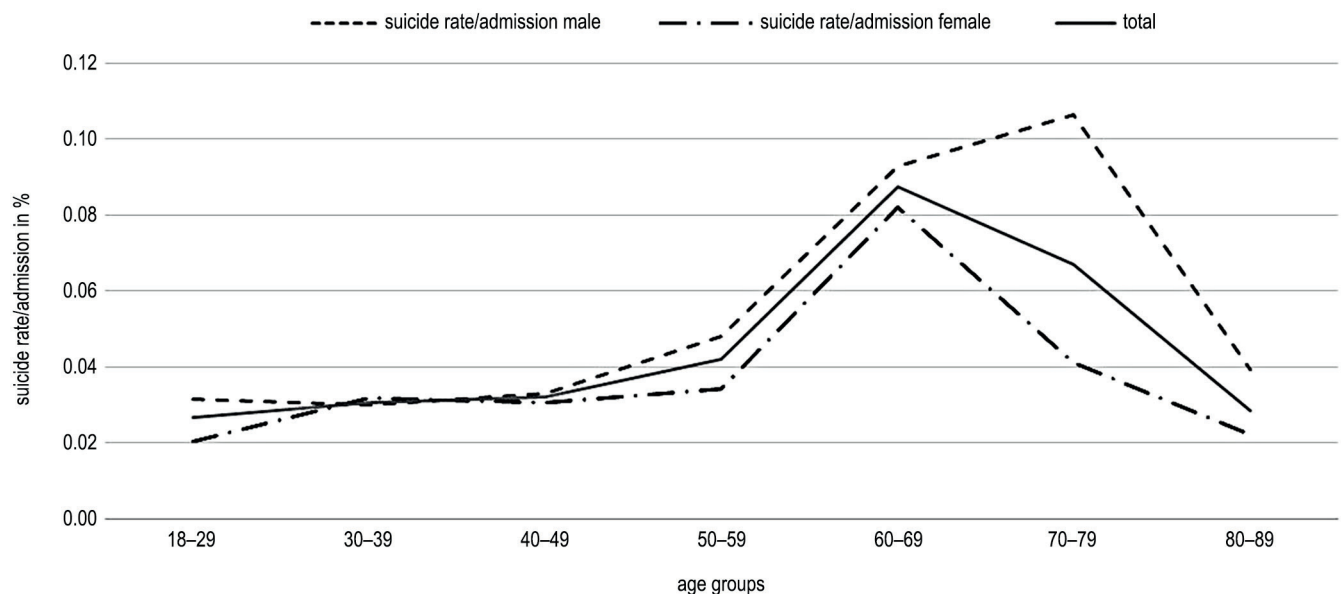


Fig. 2. Suicide rate/admission by age groups

significantly with regard to sex, overall, more men died by suicide. Of all hospitalized women and men, 0.03% and 0.04% died by suicide, respectively. Of note, in the 60–69-year age group, as many women as men died by suicide.

Diagnosis

Diagnoses were documented in 227 cases. Most patients ($n = 141$; 62.1%) suffered from affective disorders. The 2nd most common diagnosis was a psychotic disorder ($n = 65$; 28.6%). Addiction ($n = 12$; 5.3%), anxiety ($n = 6$; 2.6%), and other diagnoses ($n = 3$) accounted for only

a small proportion (Table 1). Age stratification showed a significant difference regarding diagnosis (Table 2). Post hoc tests showed that the youngest and the oldest age groups differed from the other age groups. Addiction and psychotic disorders were significantly more prevalent in the youngest age group, while affective disorders were the most prevalent diagnosis in the oldest age group.

Suicide method

Hanging ($n = 76$; 33.2%) was the most common suicide method, followed by railway suicide ($n = 40$; 17.5%) and

Table 1. Descriptive statistics of demographic variables, diagnosis, suicide method, location of suicide, possible stressors, and medication

Variables	Total, n = 229 (100.0%)	Age groups						p-value
		18–29 years, n = 30 (13.1%)	30–39 years, n = 32 (14.0%)	40–49 years, n = 44 (19.2%)	50–59 years, n = 51 (22.3%)	60–69 years, n = 38 (16.6%)	>70 years, n = 34 (14.8%)	
Demographic variables								
Female, n (%)	89 (38.9)	9 (30.0)	13 (40.6)	17 (38.6)	18 (35.3)	19 (50.0)	13 (38.2)	0.654 ^a
Age, mean (SD) [years]	50.91 (16.31)	24.84 (2.84)	35.33 (2.61)	45.20 (2.86)	54.18 (2.90)	63.97 (2.62)	76.45 (7.06)	–
Diagnoses, n	227	30*	32	44	51	38	32*	<0.001 ^a
F0, n (%)	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.1)	
F1, n (%)	12 (5.3)	4 (13.3)	1 (3.1)	2 (4.5)	4 (7.8)	0 (0.0)	1 (3.1)	
F2, n (%)	65 (28.6)	12 (40.0)	16 (50.0)	15 (34.1)	15 (29.4)	5 (13.2)	2 (6.3)	
F3, n (%)	141 (62.1)	12 (40.0)	13 (40.6)	25 (56.8)	31 (60.8)	32 (84.2)	28 (87.5)	
F4, n (%)	6 (2.6)	0 (0.0)	2 (6.3)	2 (4.5)	1 (2.0)	1 (2.6)	0 (0.0)	
F6, n (%)	2 (0.9)	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
Suicide method, n	229							0.044 ^a
Hanging, n (%)	76 (33.2)	6 (20.0)	7 (21.9)	16 (36.4)	22 (43.1)	10 (26.3)	15 (44.1)	
Fall from a great height, n (%)	34 (14.8)	3 (10.0)	5 (15.6)	9 (20.5)	7 (13.7)	9 (23.7)	1 (2.9)	
Fall from a bridge, n (%)	17 (7.4)	1 (3.3)	5 (15.6)	4 (9.1)	3 (5.9)	2 (5.3)	2 (5.9)	
Suicide by railway, n (%)	40 (17.5)	8 (26.7)	10 (31.3)	5 (11.4)	7 (13.7)	7 (18.4)	3 (8.8)	
Suicide by vehicle, n (%)	4 (1.7)	0 (0.0)	0 (0.0)	2 (4.5)	0 (0.0)	2 (5.3)	0 (0.0)	
Drowning, n (%)	6 (2.6)	1 (3.3)	0 (0.0)	1 (2.3)	0 (0.0)	1 (2.6)	3 (8.8)	
Self-injury with a sharp object, n (%)	6 (2.6)	0 (0.0)	1 (3.1)	1 (2.3)	2 (3.9)	0 (0.0)	2 (5.9)	
Intoxication with alcohol/ medication, n (%)	6 (2.6)	2 (6.7)	1 (3.1)	0 (0.0)	2 (3.9)	1 (2.6)	0 (0.0)	
Intoxication with other substances, n (%)	10 (4.4)	4 (13.3)	1 (3.1)	2 (4.5)	1 (2.0)	1 (2.6)	1 (2.9)	
Burning, n (%)	3 (1.3)	0 (0.0)	1 (3.1)	1 (2.3)	1 (2.0)	0 (0.0)	0 (0.0)	
Others, n (%)	15 (6.6)	2 (6.7)	0 (0.0)	2 (4.5)	1 (2.0)	5 (13.2)	5 (14.7)	
Unknown, n (%)	8 (3.5)	2 (6.7)	0 (0.0)	0 (0.0)	5 (9.8)	0 (0.0)	1 (2.9)	
Not documented, n (%)	4 (1.7)	1 (3.3)	1 (3.1)	1 (2.3)	0 (0.0)	0 (0.0)	1 (2.9)	0.214 ^a
Location of suicide, n	222	30	32	43	51	35	31	
On ward, n (%)	38 (17.1)	7 (23.3)	6 (18.8)	8 (18.6)	10 (19.6)	2 (5.7)	5 (16.1)	
On hospital grounds, n (%)	6 (2.7)	3 (10.0)	0 (0.0)	1 (2.3)	1 (2.0)	0 (0.0)	1 (3.2)	
At home, n (%)	56 (25.2)	3 (10.0)	3 (9.4)	9 (20.9)	16 (31.4)	12 (34.3)	13 (41.9)	
Near person's home, n (%)	5 (2.3)	1 (3.3)	1 (3.1)	2 (4.7)	0 (0.0)	1 (2.9)	0 (0.0)	
At parents' home, n (%)	6 (2.7)	1 (3.3)	1 (3.1)	1 (2.3)	2 (3.9)	1 (2.9)	0 (0.0)	
At partner's home, n (%)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)	
Outside hospital/home environment, n (%)	110 (49.5)	15 (50.0)	21 (65.6)	22 (51.2)	22 (43.1)	18 (51.4)	12 (38.7)	<0.001 ^a
Possible stressors, n	105	15	16*	21	19	20	14*	
Family conflicts, n (%)	15 (14.3)	2 (13.3)	0 (0.0)	4 (19.0)	4 (21.1)	4 (20.0)	1 (7.1)	
Partnership conflicts, n (%)	16 (15.2)	2 (13.3)	1 (6.3)	5 (23.8)	6 (31.6)	2 (10.0)	0 (0.0)	
Financial problems, n (%)	3 (2.9)	0 (0.0)	1 (6.3)	1 (4.8)	0 (0.0)	0 (0.0)	1 (7.1)	
Physical complaints and pain, n (%)	16 (15.2)	0 (0.0)	0 (0.0)	3 (14.3)	0 (0.0)	6 (30.0)	7 (50.0)	
Mental health (except for psychotic symptoms), n (%)	11 (10.5)	2 (13.3)	2 (12.5)	0 (0.0)	3 (15.8)	4 (20.0)	0 (0.0)	
Psychotic symptoms, n (%)	21 (20.0)	4 (26.7)	8 (50.0)	5 (23.8)	3 (15.8)	1 (5.0)	0 (0.0)	
Job-related problems, n (%)	4 (3.8)	0 (0.0)	2 (12.5)	0 (0.0)	1 (5.3)	1 (5.0)	0 (0.0)	
Social problems, n (%)	10 (9.5)	5 (33.3)	1 (6.3)	1 (4.8)	0 (0.0)	1 (5.0)	2 (14.3)	
Death of a close person, n (%)	5 (4.8)	0 (0.0)	0 (0.0)	1 (4.8)	1 (5.3)	1 (5.0)	2 (14.3)	
Loss of autonomy, n (%)	4 (3.8)	0 (0.0)	1 (6.3)	1 (4.8)	1 (5.3)	0 (0.0)	1 (7.1)	

Table 1. Descriptive statistics of demographic variables, diagnosis, suicide method, location of suicide, possible stressors, and medication – cont.

Variables	Total, n = 229 (100.0%)	Age groups						p-value
		18–29 years, n = 30 (13.1%)	30–39 years, n = 32 (14.0%)	40–49 years, n = 44 (19.2%)	50–59 years, n = 51 (22.3%)	60–69 years, n = 38 (16.6%)	>70 years, n = 34 (14.8%)	
Medication, n	212	27	32	41	45	37	30	
Antipsychotic medication, n (%)	122 (57.3)	16 (59.3)	19 (59.4)	24 (58.5)	30 (66.7)	19 (51.4)	14 (45.2) ^b	0.191 ^a
Antidepressant, n (%)	144 (67.3)	10 (37.0)	18 (56.3)	26 (63.4)	33 (73.3)	36 (94.7)^{c*}	21 (67.7) ^b	0.010^a
Tranquilizer, n (%)	140 (66.0)	16 (59.3)	24 (75.0)	21 (51.2)	30 (66.7)	28 (75.7)	21 (70.0)	0.347 ^a
Z-drugs, n (%)	82 (38.6)	5 (18.5)	8 (25.0)	13 (31.7)	19 (42.2)	18 (48.6)	19 (63.3)*	0.001^a
Lithium, n (%)	11 (5.2)	3 (11.1)	1 (3.1)	3 (7.3)	2 (4.4)	2 (5.4)	0 (0.0)	0.510 ^a
Medication for more than 1 indication, n (%)	68 (32.1)	4 (14.8)	5 (15.6)*	9 (22.0)	15 (33.3)	20 (54.1)*	15 (50.0)	0.002^a

Numbers present either absolute numbers plus percent of the respective age group (given in the heading) or mean plus standard deviation (SD); frequencies are given for the participants with valid data records for the respective measure; ^aX²-test (two-tailed), ^bantipsychotic and antidepressant medication was documented in 31 cases in this age group; ^cantidepressant medication was documented in 38 cases in this age group; * Significant results of the χ^2 test after Bonferroni correction, comparing each individual age group to all the others.

Table 2. Differences in diagnoses, suicide method, location of suicide, potential stressors, medication, and weekday stratified by age evaluated with the χ^2 test

Variables	n	χ^2	df	p-value	Cramér's V
Diagnoses	227	57.021	25	<0.001	0.224
18–29 years vs other age groups	227	22.715	5	0.012*	0.316
>70 years vs other age groups	227	18.119	5	0.018*	0.283
Suicide method	229	79.958	60	0.044	0.264
Location of suicide	222	35.811	30	0.214	0.180
Daycare vs inpatient unit	222	17.859	6	0.007	0.284
Possible stressors	105	79.596	45	0.001	0.389
30–39 years vs other age groups	105	21.051	9	0.03*	0.448
>70 years vs other age groups	105	26.358	9	0.003*	0.501
Medication					
Antipsychotic medication	213	19.526	15	0.191	0.175
Antidepressant	214	30.503	15	0.010	0.218
60–69 years vs other age groups	214	15.814	1	<0.001*	0.272
Tranquilizer	212	21.881	20	0.347	0.161
Z-drugs	212	30.717	10	0.001*	0.269
>70 years vs other age groups	212	8.955	1	0.024*	0.094
Lithium	212	4.276	5	0.510	0.094
Medication for more than 1 indication	212	35.810	15	0.002	0.237
30–39 years vs other age groups	212	7.529	1	0.042*	0.188
60–69 years vs other age groups	212	11.390	1	0.006*	0.232
Weekday	227	30.586	30	0.436	0.164
Daycare vs inpatient unit	227	6.654	6	0.354	0.171

df – degrees of freedom; significant p-values in bold; *significant results of the χ^2 test after Bonferroni correction, comparing each individual age group to all the others.

a fall from a great height (n = 34; 14.8%). In the age groups over 40 years, hanging was the most common suicide method, whereas in the age groups under 40 years, railway suicide was the most common suicide method. Seventeen (7.4%) patients died by jumping from a bridge and

16 (7.0%) by intoxication with drugs/alcohol or medication. Six patients died by drowning. Age stratification showed a significant difference regarding suicide method. However, the post hoc test revealed no significant differences when comparing each age group individually.

Location

The location of suicide was categorized as on the ward ($n = 38$; 17.1%), on hospital grounds ($n = 6$; 2.7%), at home ($n = 56$; 25.2%), near the person's home ($n = 5$; 2.3%), at the parents' home ($n = 6$; 2.7%), at the life partner's home ($n = 1$; 0.5%), and outside the hospital environment or home ($n = 110$; 49.5%). Age stratification showed no significant difference in location. The distribution of locations showed significant differences between the daycare unit and the inpatient unit. This can be explained by the fact that suicides on the wards or on hospital grounds were not recorded in the daycare units.

Possible stressors

In 105 cases, the physicians retrospectively discussed possible stressors with a consultant. Age stratification showed a significant difference in stressors. Psychotic symptoms were the most common stressor ($n = 21$; 20%); the proportion diminished with increasing age. In the 30–39-year age group, psychotic symptoms were assumed to be a possible stressor in half of the cases. By contrast, physical complaints and pain increased with increasing age ($n = 16$; 15.2%). Among patients aged 70 years and older, this was the assumed stress factor in 50% of cases. Post hoc tests revealed significant differences with regard to stressors when the 30–39-year age group and the oldest age groups were compared with all other age groups. In 16 cases (15.2%), partnership conflicts were discussed as possible stressors and played a major role, especially among patients aged 40–59 years. In 15 cases (14.3%), family conflicts were discussed as possible stress factors; 12 of these patients were aged 40–69 years. Mental health problems were assumed in 11 cases and social problems in 10 cases (for a detailed description of stressors by year and age, see Supplementary Table 3).

Medication

A total of 144 patients (67.3%) were prescribed at least 1 antidepressant; the percentage of patients taking antidepressants increased across the various age groups up to the 60–69-year age group and then decreased in the oldest age group. Antidepressant use differed significantly between age groups. The post hoc test showed that the 60–69-year age group differed significantly from the others. One hundred forty patients (66.0%) were prescribed at least 1 tranquilizer. The use of tranquilizers was high across all age groups and varied from 51.2 to 75.7%; there was no significant difference across all age groups. One hundred twenty-two patients (57.3%) were prescribed at least 1 antipsychotic drug; there was no significant difference across the various age groups. Eighty-two patients (38.6%) were prescribed Z-drugs; age stratification showed a significant difference. The post hoc test revealed that patients in the oldest age

group were prescribed significantly more Z-drugs than those in the other age groups. Only 11 patients (5.2%) took lithium, of whom 5 had bipolar disorder and 6 had unipolar affective disorder. Sixty-eight patients (32.1%) received medication for indications other than psychiatric ones; the percentage increased with increasing age. The post hoc test revealed that, compared with patients in the other age groups, those aged 30–39 years were prescribed significantly less medication for other indications, while those aged 60–69 years were prescribed significantly more.

Differences between hospitals

When the distribution of various parameters (sex, age groups, diagnosis, antipsychotic drugs, antidepressants, weekday, and type of leave) was compared across hospitals, only the location ($\chi^2(48; 222) = 87.369$; $p < 0.001$; Cramér's $V = 0.256$) showed a significant difference between hospitals, without this being linked to a specific location (see Supplementary Table 4).

Inpatient management

Type of leave

In 211 cases, the type of leave was documented. More than half of the patients were allowed to leave the ward for 1 day ($n = 73$; 34.4%) or on the weekend ($n = 43$; 20.2%); 31 (14.9%) patients were treated in daycare units. Forty-one patients (19.3%) were to remain on the ward. Only 23 (11.1%) patients left the ward without permission. In 17 cases, it was not documented whether the patients were allowed to leave the hospital.

Duration of hospitalization

The duration of hospitalization was known for 225 subjects who died by suicide. The duration of hospitalization, measured in days, was not normally distributed (see Supplementary Table 1). In the 1st week of treatment, 39 men and 17 women died by suicide (24.3%). Of these, 8 patients (3.6%) died on the day of admission. More than half of the suicides within the first week occurred on the ward ($n = 20$; 36.4%) or outside the hospital environment ($n = 21$; 38.2%). In the 1st month of hospitalization, more than half of the patients died (52.1%). The suicide rate by duration of hospitalization (i.e., the number of patients who died in week x divided by the total number of patients treated for x weeks) increased after the 1st month of treatment, reaching its peak in week 11 (Fig. 3).

Weekday

Fifty-nine (26.8%) patients died on Sundays and 34 (15.5%) on Saturdays (Fig. 4). If suicides on the ward were not included in the calculation, the percentage of suicides

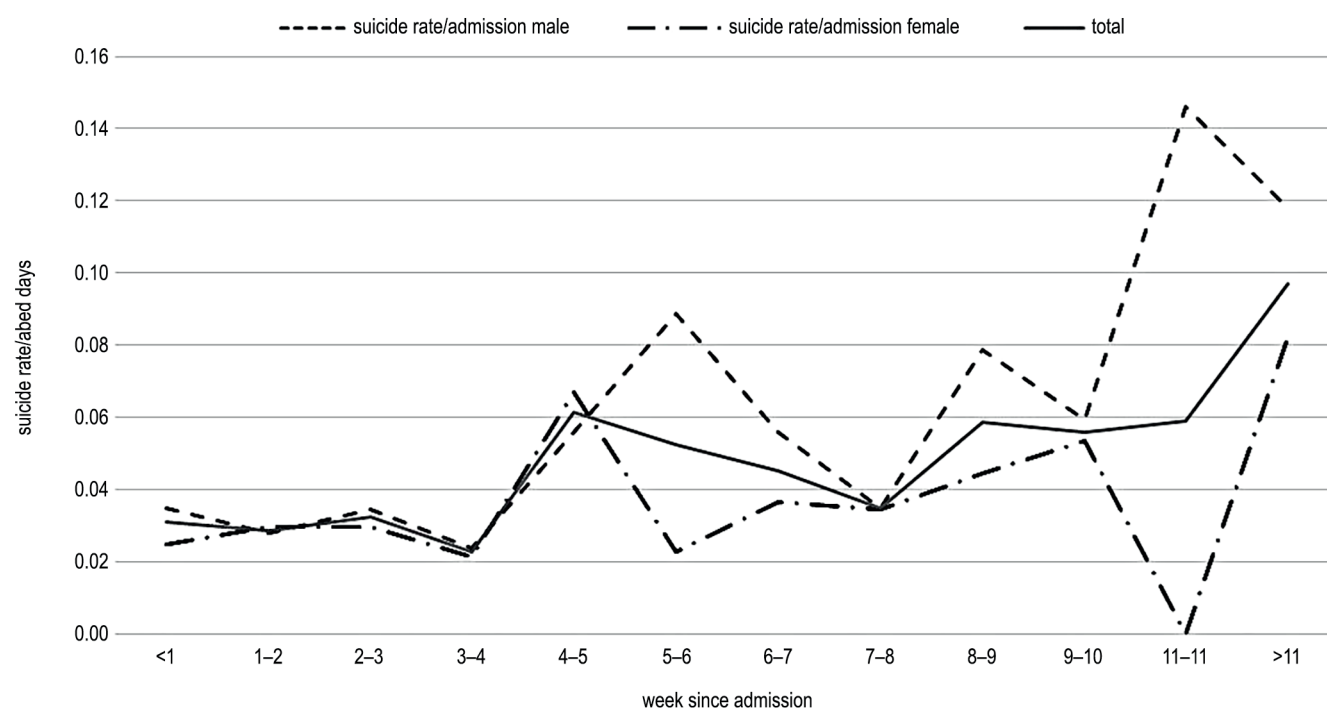


Fig. 3. Suicide rate per duration of hospitalization

on weekends increased to 43.9%. Age stratification showed no significant difference. The distribution of suicides by day of the week showed no significant differences between daycare patients and inpatients.

Suicides on the ward

A total of 38 patients died on the ward. Of these, 25 were male (65.8%), and 13 were female (the mean age was 47.1 years; SD: 16.46). The most common suicide method was hanging ($n = 34$; 89.5%). Patients often used their belts, which they fastened to doorknobs, towel rails in the bathroom, or radiators. Two patients died from self-inflicted injuries, while another died from lethal intoxication with alcohol. No approved periods of leave were documented for any of the patients. More than half of the patients ($n = 21$; 55.3%) died during the first 7 days. Twenty-four patients (63.2%) were treated for a depressive episode. In 17 (44.7%) cases, a possible stressor was speculated. Of these, 6 patients may have died by suicide due to psychotic triggers.

Discussion

The aim of this retrospective analysis was to evaluate changes in suicide rates over the years. From 2008 to 2019, the overall suicide rate ranged from 0.06% to 0.03% suicides/admission. Age-stratified analysis using the χ^2 test revealed differences in terms of sex, suicide method, diagnosis, stressors, and medication. Special features of inpatient treatment were analyzed to derive clinical considerations. Descriptive analyses showed that more than

half of the suicides occurred outside the hospital environment, on weekends, and when staff had approved a leave of absence.

Suicide rates by year

From 2008 to 2019, the overall suicide rate was 39.50 per 100,000 admissions (0.04%). The suicide rate per admission for men was higher than that for women in all years except for 2008. The overall suicide rate was 43.4 per 100,000 admissions (0.04%) for men and 34.5 per 100,000 admissions for women (0.03%; Fig. 1). The suicide rate was highest in 2017 (0.05%). In this study, the overall suicide rate seemed stable throughout a decade, and no decrease was observed. These results are in contrast to others.^{18–20} The meta-analysis by Walsh et al.⁴ even revealed an increase in hospital suicide rates over the last 3 decades. There is controversy over whether a reduction in hospital beds is associated with an increase in inpatient suicide rates.^{20,21}

Age differences

A systematic review showed that several studies demonstrated an increased risk of suicide in men compared to women.²² In contrast, a meta-analysis found no significantly increased risk for men.²³ In the 2 largest studies cited in the review, with samples of 279²⁴ and 222²⁵ suicides, about 60% of the patients were male, a proportion similar to that in this sample. The risk estimates were 2.58 and 1.49. In both studies, there was no age stratification with regard to sex.^{24,25} A nonsignificant result in other

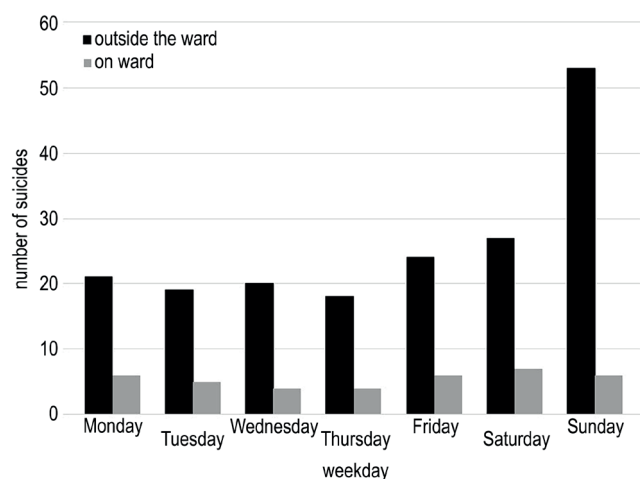


Fig. 4. Distribution of suicides per weekday

studies could be due to different age groups. Our study shows that the proportion of women who died by suicide increased in the 60–69-year age group compared to that of men. Neither Neuner et al.⁸ nor Powell et al.²⁶ identified male sex as a risk factor. The mean ages of their samples were several years higher than the mean age of the sample studied by Hunt et al.²⁵ A meta-analysis revealed that both affective disorders and schizophrenia are risk factors for inpatient suicide with odds ratios (ORs) of 1.93 and 2.48, respectively.²³ Most patients were diagnosed with a depressive disorder, followed in frequency by a psychotic disorder, which is in line with findings from other studies.^{24,27,28} In another study, however, more patients with schizophrenia died by suicide, maybe because the mean age was 35.4 years.²⁹ Another reason could be that this is an older study. Wolfersdorf et al. reported a shift in diagnoses in the 1990s.¹⁸ Our results show that younger patients were more likely to suffer from psychotic disorders, whereas older patients were more likely to suffer from a depressive disorder. These results were confirmed by the post hoc tests. Only 12 patients were diagnosed with a primary substance use disorder. However, there is still an increased risk of suicide due to addiction, especially in cases of comorbid affective or psychotic disorders.³⁰

Antidepressant intake is a risk factor for inpatient suicide, whereas antipsychotic drug intake is not a significant risk factor.²³ Our results show that a high proportion received both antidepressant and antipsychotic medication and that tranquilizers were prescribed for 2/3 of patients. This could indicate that the patients had more severe or even therapy-resistant depressive or psychotic symptoms. The frequent prescription of tranquilizers could also indicate that the patients often showed anxious and agitated psychopathology. Both treatment-resistant⁸ and anxious/agitated symptoms²³ are discussed as risk factors for suicide. Of note, only a few patients in this population received lithium, although lithium has been shown to have an anti-suicidal effect.^{31,32} With increasing age, the prescription of Z-drugs increased in this sample. A large

longitudinal case-control cohort study of late-life suicide showed that poor subjective sleep quality is associated with an increased risk of death by suicide 10 years later.³³

Hanging is the most frequently reported suicide method,³⁴ followed by intoxication or jumping from a great height/in front of a moving vehicle.^{24,25,34} In our analysis, hanging was the most common suicide method, followed by railway suicide and jumping from a great height. We also included the subcategory of railway suicides, as this method of suicide was particularly prevalent among younger age groups, whereas hanging was the most common suicide method in older age groups. Particular attention must be given to the patients who died on the ward. Here, hanging (89.5%) was clearly the predominant suicide method, which underlines the importance of preventive room equipment and hospital design.^{19,34,35} Although our data are descriptive in nature, studies by others have demonstrated that preventive construction can reduce suicide rates.^{34,35} Figure 5 shows examples of suicide-preventive structures. In 53.8% of those cases, a door, door handle, or door hinge was used as a ligature fixation point.³⁴ A meta-analysis on lethality showed that hanging was the 2nd most lethal suicide method after the use of firearms.³⁶ Schmidtke et al. reported a tendency toward more lethal suicide methods in old age compared with the 1980s, with more women dying by jumping from a height and more men dying by suicide using firearms. The predominant suicide method was hanging, which did not change over time.³⁷ In accordance with this study, only a few patients died by intoxication. For the younger age groups, railway suicide was the most common suicide method. After the suicide of the famous German football player Robert Enke, an increase in railway suicides was observed in Germany.^{38,39} Special safety construction for railway tracks seems to have political implications, particularly with regard to suicide prevention.^{35,40} Risk factors for inpatient railway suicides were a change of therapist, suicidal ideation, a negative or unchanged therapeutic course, and the need for polypharmaceutical treatment.⁴¹

In 105 cases, a possible stressor was retrospectively assumed. For young patients, psychotic symptoms were the most important stressor in this sample, whereas physical complaints and pain were the most important stressor for the older population. In accordance with our results, chronic physical disease and subjective health status were risk factors for suicidal ideation in the population aged 60 years and older.^{42–45} Partnership and family conflicts were the most relevant stressors for the middle age groups. Waern et al. showed that family conflict, serious physical illness, loneliness, and both major and minor depression were associated with suicide in seniors aged 75 years and older.⁴⁶ Bowers et al. revealed that the location and time of suicide were associated with the absence of support and supervision and with the presence of family conflict.⁴⁷ They underscored the importance of addressing family conflicts as part of the therapeutic process and

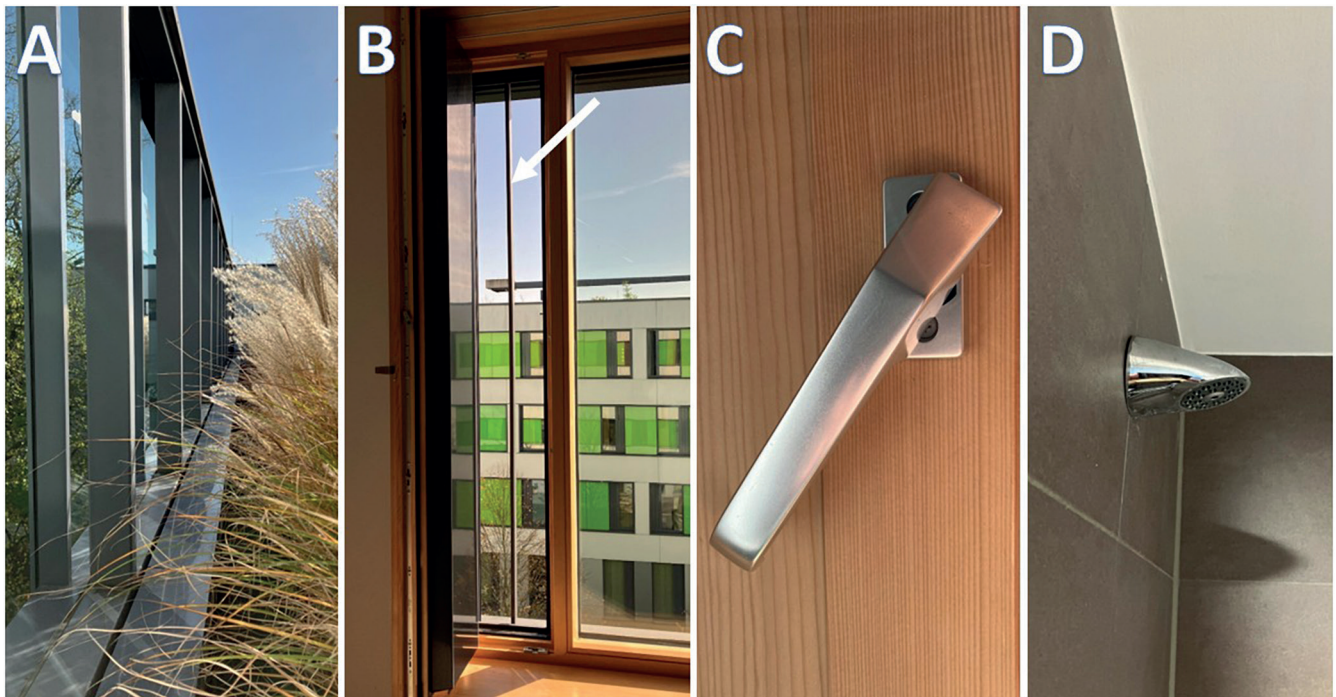


Fig. 5. Suicide-preventive architecture and room equipment. A. A roof-top sidewall to prevent climbing over; B. A window with a vertical strut (arrow) to prevent exiting; C. A window handle to prevent ropes being fixed; D. A shower head to prevent ropes being fixed

of preventing the isolation of patients behind closed doors. Furthermore, treatment of somatic comorbidities and pain should be integrated into psychiatric treatment.⁴⁸

Inpatient management

Two important aspects for inpatient treatment can be derived from this retrospective analysis. First, 24.3% of suicides occurred within the 1st week of treatment. In the 1st month of treatment, 52.3% of patients died, which is in accordance with findings from other studies.²⁵ Second, almost half of the patients died by suicide on weekends (42.3%). Of the 229 suicides, 110 suicides happened outside the hospital environment or home, 56 happened at the patient's home, and 38 on the ward. Only 17 patients left the ward without approval. However, the suicide rate by duration of hospitalization increased after the 1st month of treatment, which may highlight the severity of the underlying mental illness, resulting in a longer hospitalization period and potential therapy resistance. Despite the descriptive nature of the analysis, patients should be carefully monitored, and approved periods of leave should be considered with caution.^{23,25} In addition, patients with psychotic symptoms are especially endangered.²⁴

Limitations of the study

Several limitations must be considered in this retrospective data analysis. The protocol sheets under investigation covered only a few clinical parameters. As mentioned, possible stressors which may have influenced the suicidal act

were discussed and documented only in retrospect as part of a supervision measure for the treating physicians. However, given that a suicide represents a serious critical incident and that supervision normally takes place within 2 weeks following a suicide and includes a review of the medical record, memory lapses seem unlikely. Although the exact time of the suicide would also be of interest, data were shown to be highly inconsistent, since in many cases a precise time of death could not be determined. It is very difficult to find suitable control groups for studies on suicide. Here, we only present case studies. As data on bed days spent on weekends for all patients and denominators for diagnoses were unavailable, the results remain descriptive. Nine hospitals were included in this analysis. These hospitals may differ in various areas, but our analysis showed that they did not differ significantly in terms of gender, age, diagnosis, medication, or day of the week. Only location showed a significant difference between hospitals, though this was not linked to a specific location. Data on possible stressors were incomplete. However, the inclusion of 9 hospitals enabled us to gather information on 229 suicides. Daycare involves daily community exposure, which could affect comparability between daycare patients and inpatients. For this reason, special consideration was given to these patients with regard to leave policies.

Conclusions

The overall suicide rate among inpatients did not decrease over a decade in this population. Thus, preventive measures to protect psychiatric inpatients from suicide attempts remain

highly relevant. Differences in possible stressors, diagnoses, and methods of suicide were depicted between the age groups. Age stratification gives important insights into differences in inpatient suicides. In the younger age groups, railway suicide was the most common suicide method, whereas hanging was the most common suicide method in the older age groups. Most patients suffered from affective disorders; the proportion increased with increasing age. Potential stressors were physical complaints and pain. Suicides often occurred on weekends and during approved periods of leave outside the patient's familiar environment. Therefore, approved periods of leave should be carefully monitored.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.18661145>. The package contains the following files:

Supplementary Table 1. Results of the Kolmogorov–Smirnov test.

Supplementary Table 2. Number of admissions by year and age group.

Supplementary Table 3. Stressors in relation to year and hospital.

Supplementary Table 4. Distribution of locations across nine psychiatric hospitals.

Data Availability Statement

The datasets generated and/or analyzed during the current study are not publicly available due to ethical issues involving participants' data and privacy but are available from the corresponding author on reasonable request.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Herpes zoster epidemiology during and after the COVID-19 pandemic: A 5-year retrospective study from primary care in Poland

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Abstract

Background. Herpes zoster (HZ) is caused by reactivation of latent varicella-zoster virus and affects 25–30% of individuals, with the highest incidence in adults aged ≥ 50 years and a lifetime risk exceeding 50% after the age of 85. Its most common complication is postherpetic neuralgia (PHN), i.e., persistent neuropathic pain. Reports of increased HZ incidence during or after SARS-CoV-2 infection suggest potential epidemiological associations requiring further investigation.

Objectives. Our goals were to evaluate the incidence of HZ and its complications during the pandemic and the post-pandemic period in primary care settings in Poland.

Materials and methods. We conducted a retrospective multicenter study in 20 primary care clinics in Poland, covering 43,949–81,091 patients from 2020 to 2024. Incident HZ cases and complications (PHN, herpes zoster ophthalmicus (HZO), HZ oticus, and disseminated HZ) were identified, and demographic data, including place of residence, were collected. Statistical analyses included χ^2 or Fisher's exact tests, Student's t-tests or nonparametric equivalents, odds ratios (ORs) with 95% confidence intervals (95% CIs), and receiver operating characteristic (ROC) analysis to assess age-related risk of prolonged symptoms.

Results. A total of 570 HZ cases were recorded from 2020 to 2024. Incidence ranged from 1.61 to 2.14 per 1,000 persons and increased with age, reaching 6.57 per 1,000 in individuals aged ≥ 80 years. Patients aged ≥ 71 years had higher odds of developing PHN (pain persisting for >90 days) than younger patients (OR = 4.47; 95% CI: 2.30–8.67; $p < 0.001$). Older adults were also more likely to develop HZO ($p = 0.001$). Rural incidence was significantly lower during the pandemic than in the post-pandemic period ($p = 0.005$), with no significant differences in urban or semi-urban areas.

Conclusions. The risk of HZ, PHN, and HZO rises with age. During the pandemic (2020–2021), rural incidence was significantly lower than in the post-pandemic period (2022–2024). The study provides updated data on HZ epidemiology in Poland and highlights the impact of the COVID-19 pandemic on healthcare access and HZ reporting in rural areas.

Key words: herpes zoster, postherpetic neuralgia, herpes zoster ophthalmicus, COVID-19 pandemic, primary healthcare

Highlights

- Herpes zoster (HZ) incidence increases markedly with age, with the highest rates observed in adults aged 80 years and older.
- Older age is strongly associated with an increased risk of postherpetic neuralgia (PHN) and HZ ophthalmicus in patients with HZ.
- A multicenter primary care study provides updated epidemiological data on HZ and its complications in Poland from 2020 to 2024.
- The COVID-19 pandemic influenced HZ reporting patterns, with significantly lower incidence observed in rural areas during the pandemic compared with the post-pandemic period.

Background

Herpes zoster (shingles; HZ) is caused by reactivation of the latent varicella-zoster virus (VZV), which persists in the sensory ganglia after primary infection.¹ Primary infection with VZV – varicella – occurs predominantly in childhood, manifests as a vesicular, itchy rash accompanied by systemic symptoms such as malaise, headache, or loss of appetite, and is highly contagious.^{2–9}

Approximately 25–30% of the general population will develop HZ during their lifetime, with the highest incidence observed in adults aged ≥ 50 years.¹⁰ The risk rises to more than 50% in patients aged >85 years.^{11,12} Clinically, HZ manifests as an erythematous vesicular rash in 1 or 2 dermatomes, most commonly facial or thoracic,¹³ but it may involve multiple dermatomes or become disseminated, particularly in immunocompromised patients.^{14,15} Acute pain typically resolves within 2–4 weeks; however, more than 20% of individuals develop postherpetic neuralgia (PHN),^{16–18} with the incidence increasing substantially with age.^{19–25}

Postherpetic neuralgia is described as aching, burning, throbbing, or shooting pain and may involve allodynia.²⁶ Herpes zoster ophthalmicus (HZO) affects the ophthalmic branch of the trigeminal nerve and may lead to conjunctivitis, keratitis, uveitis, or vision loss,^{27,28} while HZ oticus can result in facial palsy, vertigo, tinnitus, or hearing loss.^{29,30} Herpes zoster occurs globally, with no apparent seasonal variation in its incidence rate (IR). The clinical and epidemiological characteristics of HZ are largely consistent across different geographic regions.³¹

Growing evidence suggests that the coronavirus disease 2019 (COVID-19) pandemic has influenced the epidemiology and reporting of non-COVID infectious diseases, including HZ. Reduced access to routine medical care and changes in healthcare-seeking behavior were widely documented during the pandemic, leading to underdiagnosis and fluctuations in surveillance data.^{32,33}

Additionally, case series and observational studies have described VZV reactivation during or following infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), suggesting that pandemic-related immune

dysregulation or physiologic stress may contribute to triggering HZ in susceptible individuals.^{34,35} These observations highlight the importance of evaluating pandemic-related trends in HZ and PHN incidence.

In Poland, vaccination against varicella is included in the mandatory childhood immunization schedule, while vaccination against HZ is recommended for adults but not universally funded. Since 2024, recombinant zoster vaccine (RZV) has been partially reimbursed for selected adult groups, although availability remains limited and vaccination is not routinely offered in primary care settings.³⁶ These factors underline the public health relevance of analyzing HZ epidemiology and its complications in the Polish population, including differences between urban and rural settings.

The Podkarpackie Voivodeship provides a valuable setting for such an analysis because of its mixed urban–rural structure, aging population, and documented disparities in healthcare accessibility relative to other regions of Poland. Primary care practices in this area provide access to large community-based patient populations, enabling the assessment of HZ incidence and meaningful comparisons across demographic and geographic subgroups.

Objectives

Considering the well-established risk factors for HZ reported worldwide^{37–39} and the limited epidemiological data available from Central and Eastern Europe, this retrospective, population-based study aimed to characterize the epidemiology of HZ in Poland between 2020 and 2024. Specifically, we determined the incidence of HZ and its associated complications, including PHN, HZO, HZ oticus, and disseminated HZ, with particular attention to differences between urban and rural populations. We also examined the influence of age and sex on HZ incidence and assessed the impact of the COVID-19 pandemic by comparing data from the pandemic period (2020–2021) with those from the post-pandemic years (2022–2024), while accounting for territorial differences.

Materials and methods

Study population

We conducted a retrospective, population-based study using the primary care database of Medical Center Medyk in the Podkarpackie Voivodeship, Poland. Podkarpackie is the southeasternmost voivodeship in the country, and Medical Center Medyk is one of the largest multisite healthcare providers in the region, operating 20 primary care clinics across the area. The total number of patients registered with a general practitioner increased from 43,949 at the end of 2020 to 81,091 at the end of 2024 (Table 1). This substantial growth resulted primarily from the ongoing registration of new patients, reflecting the gradual enlargement of the study population over the study period. In this study, we analyzed all cases of HZ (ICD-10: B02–B02.9) diagnosed in outpatient primary care settings over a 5-year period (January 2020–December 2024). The database was used to identify patients with HZ and its complications, including PHN, HZO, HZ oticus, and disseminated HZ.

Urban areas were defined as cities with more than 100,000 inhabitants, suburban areas as towns with municipal status but fewer than 100,000 inhabitants, and rural areas as non-urbanized localities without municipal rights, regardless of population size. The IR is a measure of HZ incidence that expresses the number of new cases that arise in a study population during a given year.

Comorbidities were not included in the analysis because the documentation in the primary care database was insufficient. Many chronic conditions are managed exclusively by specialists rather than documented in primary care records, resulting in incomplete records that do not allow for a robust evaluation of their potential impact. Vaccination rates for HZ were not addressed in this study, as RZV has been partially reimbursed in Poland since January 2024 for individuals aged ≥ 65 years with comorbidities and has been fully reimbursed since April 2025 for this population (https://bip.aotm.gov.pl/assets/files/zlecenia_mz/2024/158/AWA_158_AWA_OT.423.0.7.2024_Shingrix_BIP_REOPTR.pdf). Before January 2024, the vaccine was available only commercially, with uptake generally very low.⁴⁰

Table 1. Total number of patients by year

Year	Number of patients with HZ	Total number of patients	Incidence per 1,000 patients per year
2020	94	43,949	2.139
2021	95	50,976	1.864
2022	124	61,958	2.001
2023	126	71,736	1.756
2024	131	81,091	1.616

HZ – herpes zoster.

Definition of cases and complications

An incident case of HZ was defined as a new diagnosis (ICD-10: B02) recorded between 2020 and 2024 in individuals with no prior HZ diagnosis in the preceding 12 months. The data extracted from the Medical Center Medyk primary care database were checked for completeness and consistency to ensure reliable identification of HZ cases and complications. Postherpetic neuralgia was identified based on either the co-occurrence of ICD-10 codes B02 and G53.0 or B02 and G63.0, recorded on the same date more than 90 days after the initial HZ diagnosis and listed as primary or secondary diagnoses, or documentation of persistent postherpetic pain lasting more than 90 days in the medical history. Other HZ-related complications were defined and identified analogously using specific 5-character ICD-10 codes (B02.0–B02.9) corresponding to specific clinical manifestations. These included, e.g., HZO (B02.3), HZ oticus (B02.2), and disseminated HZ (B02.7). All diagnoses were further verified by trained personnel to ensure consistency and minimize misclassification. Detailed medical history entries recorded within 30 days of the initial HZ diagnosis were also reviewed to ensure that complications not fully reflected in the diagnostic codes were accurately captured.

Approval for the study was granted by the Bioethics Committee at the University of Rzeszów, Poland (approval No. 044/11/2024, issued on 6 November 2024), and all consent required by the Committee was obtained.

Statistical analyses

We used TIBCO STATISTICA v. 13.3 software (StatSoft Inc., Tulsa, USA) and R v. 4.3.1 (R Foundation for Statistical Computing, Vienna, Austria; packages: stats, base, car 3.1-5, FSA 0.10.1, pROC 1.19.0.1, and sm 2.2-6.0; base: <https://search.r-project.org/R/refmans/base/html/base-package.html>; stats: <https://search.r-project.org/R/refmans/stats/html/stats-package.html>; car 3.1-5: <https://CRAN.R-project.org/package=car>; FSA 0.10.1: <https://CRAN.R-project.org/package=FSA>; pROC 1.19.0.1: <https://cran.r-project.org/web/packages/pROC/index.html>; sm 2.2-6.0: <https://cran.r-project.org/web/packages/sm/index.html>) to analyze the data. Categorical variables were presented as numbers with percentages and compared using the χ^2 test or Fisher's exact test, as appropriate. The Shapiro–Wilk ($n = 10–50$) or Lilliefors ($n > 50$) test was used to assess the normality of the data distribution (the results are presented in Supplementary Table 2). Medians with Q1–Q3 ranges and means with 95% confidence intervals (95% CIs) and standard deviations (SDs) were presented for all continuous variables. As the assumptions for parametric tests were not met (Supplementary Table 2), the Mann–Whitney or Kruskal–Wallis test was used, as appropriate, to compare continuous variables between study groups. For multiple comparisons, Tukey's post hoc test with Bonferroni correction or Dunn's

Table 2. Demographic distribution of herpes zoster cases by year (2020–2024): number of cases (n) and incidence per 1,000 persons per year (incidence rate (IR))

Variable	2020		2021		2022		2023		2024	
	n	IR	n	IR	n	IR	n	IR	n	IR
Age [years]										
0–17	2	0.463	1	0.157	8	0.856	2	0.174	2	0.149
18–49	23	1.048	30	1.174	42	1.345	39	1.066	37	0.882
50–59	22	3.706	17	2.631	21	2.847	25	3.047	24	2.682
60–69	27	4.872	20	3.366	27	4.064	26	3.542	32	4.027
70–79	14	3.332	17	3.761	16	3.180	17	3.083	21	3.532
>80	6	3.000	10	4.695	10	4.294	17	6.571	15	5.212
Sex										
Women	63	2.747	66	2.482	72	2.193	79	2.074	78	1.813
Men	31	1.475	29	1.189	52	1.785	47	1.397	53	1.393
Area of living										
Rural	0	0.000	7	2.928	11	4.207	15	3.608	11	2.213
Suburban	12	1.297	14	1.230	21	1.540	13	0.831	24	1.357
Urban	82	2.519	74	1.989	92	2.013	98	1.887	86	1.472
Total	94	2.139	95	1.864	124	2.001	126	1.756	131	1.615

test, as appropriate, was used. Spearman's correlation coefficient was used to describe the relationship between quantitative characteristics. The test of monotonicity in a regression curve (sm.monotonic) was used to assess monotonicity. This test uses the concept of a "critical bandwidth" to assess evidence that a regression curve is non-monotonic. A two-proportion test was used to compare the IR. For binary variables, odds analysis (with a 95% CI) was used to estimate the odds ratio (OR), with a significance level of $p = 0.050$. Receiver operating characteristic (ROC) analysis was used to determine the age that best discriminated between patients with pain lasting >90 days and those with pain lasting ≤90 days. A two-sided $p < 0.05$ was considered statistically significant.

Results

Herpes zoster was diagnosed in 570 individuals over the 5-year period (2020–2024). The demographic distribution of HZ cases by year, along with the IR, is presented in Table 2, whereas IRs with corresponding 95% CIs are summarized in Supplementary Table 1.

Table 3 presents the median time between the first and last visits and the number of visits during the course of illness, along with the corresponding 1st and 3rd quartiles for each year.

The incidence by age group is shown in Fig. 1. The incidence of HZ by sex from 2020 to 2024, expressed per 1,000 persons per year, is summarized in Fig. 2. Supplementary Fig. 1 presents the IR across urban, semi-urban, and rural areas.

Descriptive statistics for age and time to the last visit according to HZ localization are summarized in Table 4.

Table 3. Descriptive statistics of the number of visits, pain duration, patient age, and time between first and last visit during the pandemic and post-pandemic periods

Year	Number of patients with HZ	Median	Q1	Q3
2020	94	1	1	2
2021	95	1	1	2
2022	124	1	1	2
2023	126	1	1	2
2024	131	1	1	2
Pain duration [days]				
2020	94	0.00	0.00	15.00
2021	95	0.00	0.00	14.00
2022	124	0.00	0.00	20.00
2023	126	0.00	0.00	11.00
2024	131	0.00	0.00	21.00
Pain duration [days] broken down into 90 days				
Group	n in group	median	Q1	Q3
≤90	531	58.00	43.00	69.00
>90	39	72.00	60.00	78.00
Descriptive statistics of age of patients with HZ in pandemic and post-pandemic periods				
Years	n in years	median	Q1	Q3
2020–2021	189	59.000	46.000	69.000
2022–2024	381	58.000	43.000	70.000
Time between first and last visit in the pandemic and post-pandemic periods [days]				
Year	n in years	median	Q1	Q3
2020–2021	78	27	7	56
2022–2024	156	32	8	74

HZ – herpes zoster; Q1 – 1st quartile; Q3 – 3rd quartile

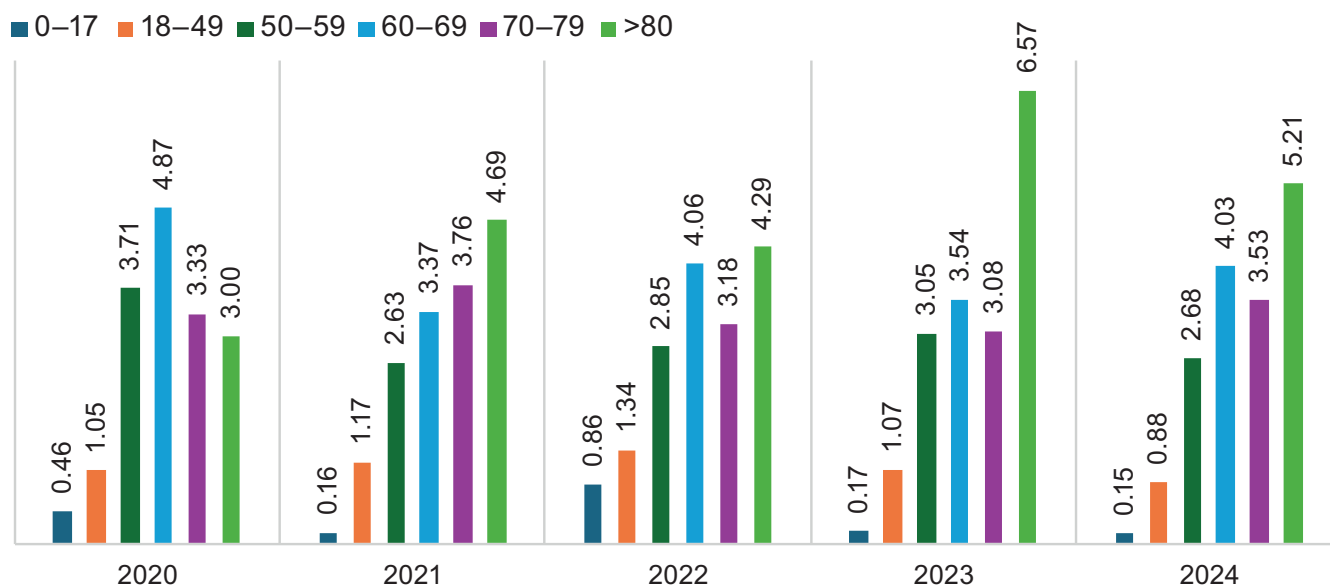


Fig. 1. Incidence by age group per 1,000 persons per year in 2020–2024

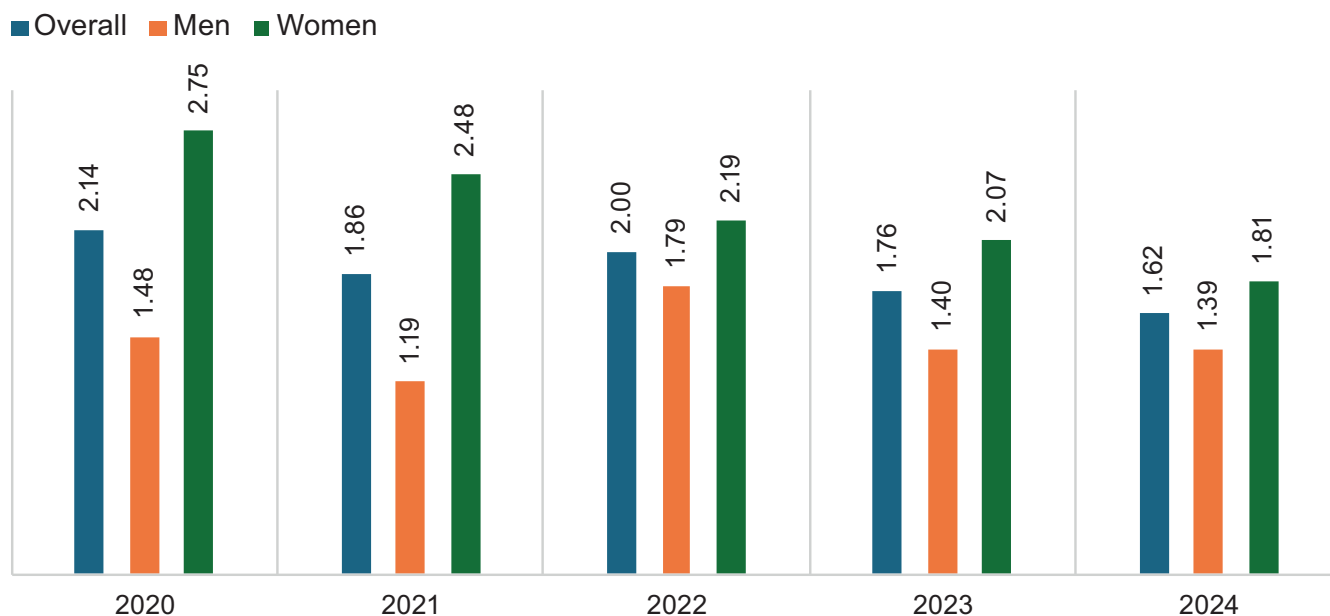


Fig. 2. Incidence by sex group per 1,000 persons per year in 2020–2024

Table 4. Descriptive statistics of age and time to last visit according to herpes zoster (HZ) localization

Localization	Variable	n	Median	Q1	Q3
HZ ophthalmicus	age	32	69	60	78
	time to last visit	32	3.5	0	40.5
HZ oticus	age	13	63	51	70
	time to last visit	13	3	0	70
Limited HZ	age	485	58	43	69
	time to last visit	485	0	0	16
Localization	variable	n	–	min	max
Disseminated HZ	age	3	–	42	73
	time to last visit	3	–	0	101

Q1 – 1st quartile; Q3 – 3rd quartile; min – minimum; max – maximum.

In 37 cases (6.49%), information on HZ localization was unavailable. To examine whether patient age differed significantly across HZ types, the nonparametric Kruskal–Wallis test was applied because some subgroups had small sample sizes. The Kruskal–Wallis test showed that age differed significantly across HZ localization groups ($n = (32, 13, 3, 485)$; $p < 0.001$), whereas time to the last visit did not ($n = (32, 13, 3, 485)$; $p = 0.222$). Patients with HZO were significantly older ($p < 0.001$) than patients with a limited form of HZ. No significant age differences were observed in the other pairwise comparisons (ophthalmicus vs oticus: $p = 0.915$; ophthalmicus vs disseminated: $p > 0.999$; oticus vs limited: $p > 0.999$; oticus vs disseminated: $p > 0.999$; ophthalmicus vs limited: $p > 0.999$; disseminated vs limited: $p > 0.999$).

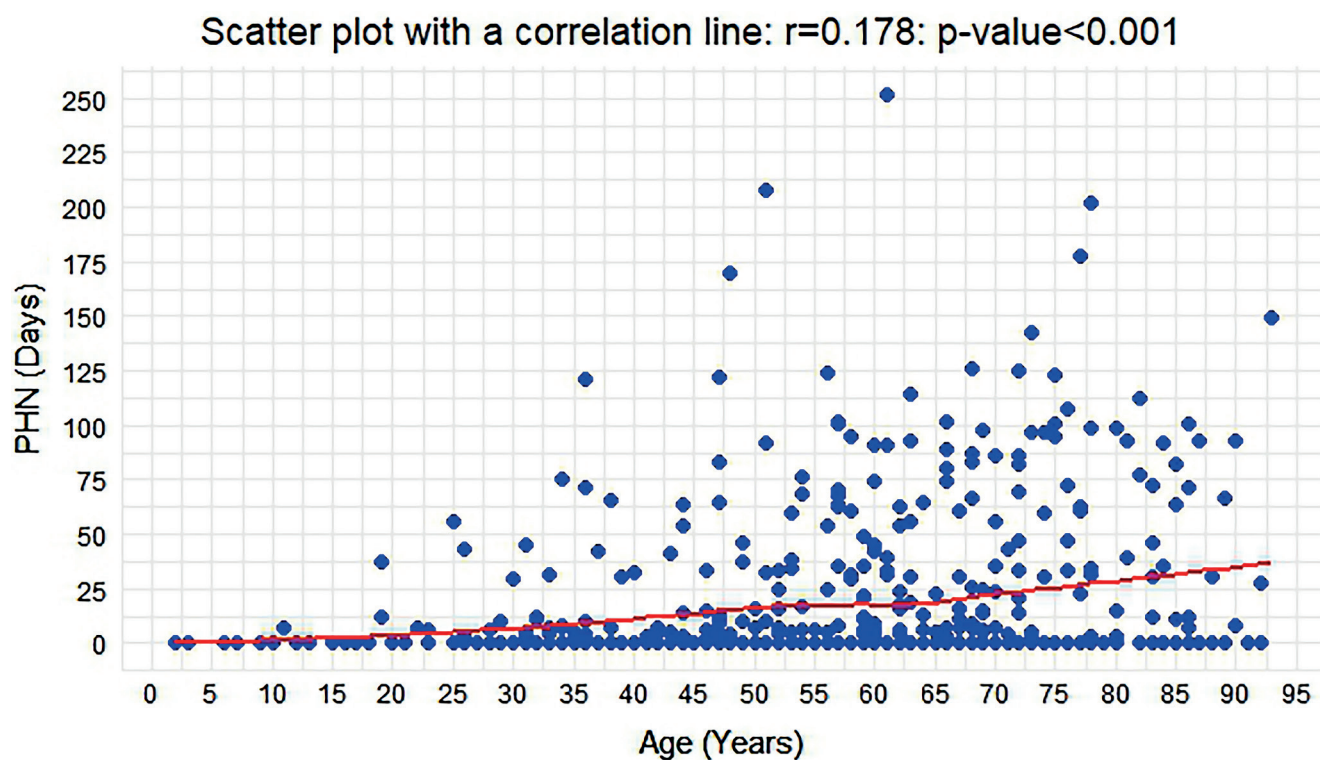


Fig. 3. Association between age and postherpetic neuralgia (PHN) risk

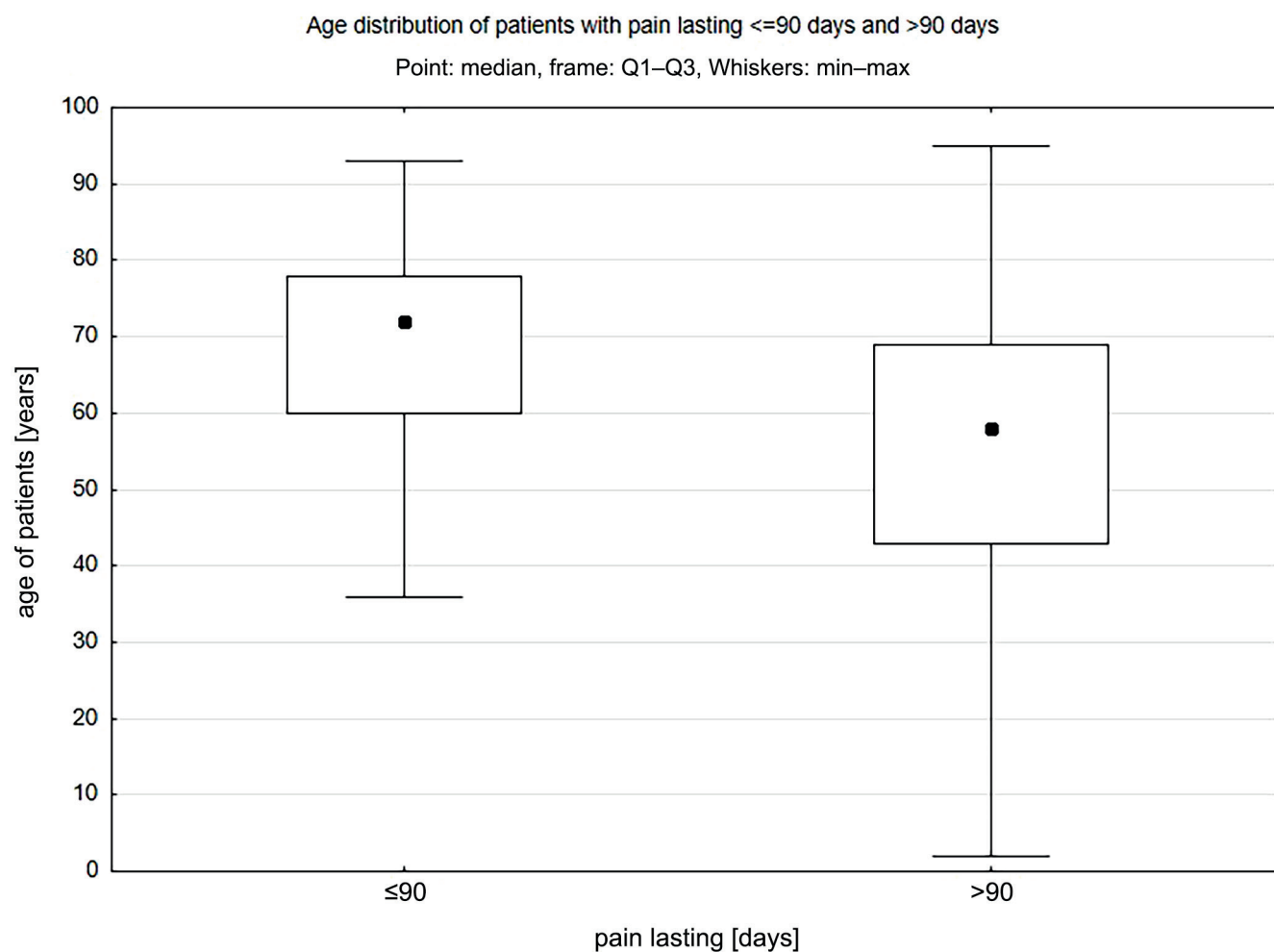


Fig. 4. Age distribution of patients with pain lasting ≤ 90 days and >90 days (postherpetic neuralgia (PHN))

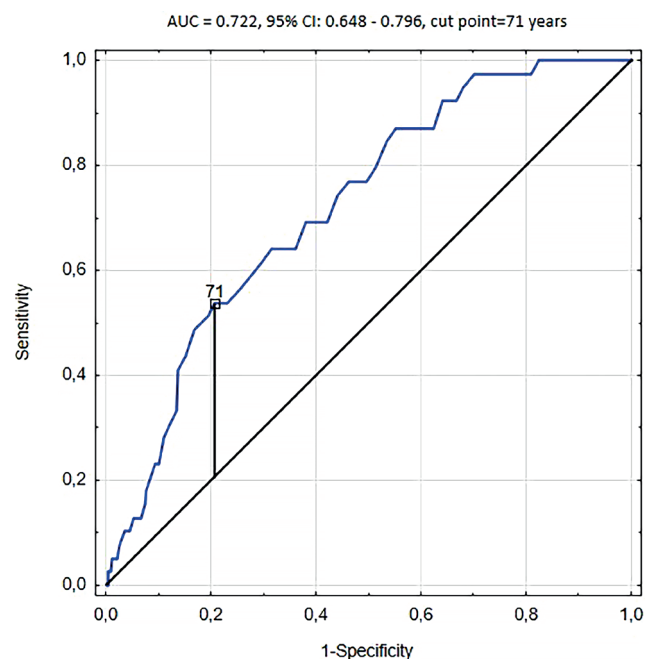


Fig. 5. Receiver operating characteristic (ROC) curve evaluating the performance of age as a predictor of prolonged pain duration (>90 days)

Thirty-nine patients (7%) developed PHN, defined as chronic pain lasting more than 90 days. Spearman's correlation analysis showed a significant positive association between age and PHN ($r = 0.178$, $n = 570$; $p < 0.001$, $p\text{-sm.monotonic} = 0.380$) (Fig. 3). When the duration of postherpetic pain was treated as a binary variable (≤ 90 days vs > 90 days), the Mann–Whitney U test revealed that patients with PHN were significantly older ($n = (39, 531)$; $p < 0.001$). Table 3 presents the descriptive statistics for pain duration and the results of the Mann–Whitney U test, while Fig. 4 illustrates the age distribution of patients with pain lasting ≤ 90 days vs those with pain persisting for > 90 days. Receiver operating characteristic analysis identified an optimal age cutoff of 71 years (area under the ROC curve (AUC) = 0.722, 95% CI: 0.648–0.796; $p < 0.001$; Fig. 5). Patients aged ≥ 71 years had 4.47 times the odds of developing PHN compared with patients aged < 71 years (OR = 4.47, 95% CI: 2.30–8.67; $p < 0.001$).

Impact of the COVID-19 pandemic on the burden of herpes zoster

The incidence of HZ during the pandemic period (2020–2021) and the post-pandemic period (2022–2024) is shown in Table 5. The average annual rate of change from 2020 to 2024 was -6.78% . The duration of HZ symptoms, as well as patient age and sex, did not differ significantly between the pandemic and post-pandemic periods. The median and mean age, as well as the mean time between the first and last HZ-related visits, are presented in Table 3 and Supplementary Fig. 2. As most patients attended only a single visit, the median interval between the first and last visits was 0 in both the pandemic and post-pandemic periods. The incidence of HZO, HZ oticus, and disseminated HZ did not differ significantly between these 2 periods (Mann–Whitney U test, $n = (189, 381)$; $p = 0.761$).

The incidence of HZ differed significantly between the pandemic and post-pandemic periods in rural areas (two-proportion test: $f = (1.542, 3.151)$; $p = 0.005$), but not in urban (two-proportion test: $f = (2.236, 1.769)$; $p = 0.452$) or semi-urban areas (two-proportion test: $f = (1.260, 1.235)$; $p = 0.952$) (Supplementary Fig. 3). Overall HZ incidence did not differ significantly between the 2 periods (two-proportion test: $f = (1.991, 1.774)$; $p = 0.717$).

Discussion

This study provides a comprehensive analysis of HZ epidemiology and its complications in the Polish population. We confirmed that HZ incidence increases markedly with age, that women are affected more frequently than men, and that PHN remains the most common complication, with the risk strongly associated with age. Although overall HZ incidence did not differ significantly between urban and rural areas, reporting patterns varied during the pandemic period. These findings are consistent with international data and provide important evidence from Central and Eastern Europe, where population-based analyses remain limited.

Table 5. Incidence of herpes zoster (HZ) in the pandemic (2020–2021) and post-pandemic (2022–2024) periods

Period	Years	Number of patients with HZ	Total number of patients	Incidence per 1,000 persons per year	Mean of incidence per 1,000 persons per year in periods
Pandemic	2020	94	43,949	2.14	2.00
	2021	95	50,976	1.86	
Post-pandemic	2022	124	61,958	2.00	1.79
	2023	126	71,736	1.76	
	2024	131	81,090	1.62	

Impact of the COVID-19 pandemic on HZ diagnosis and healthcare access

Overall, HZ incidence did not differ significantly between the pandemic and post-pandemic periods in our study, but rural areas showed a lower incidence during the pandemic, likely reflecting reduced healthcare access, diagnostic delays, and underreporting during the pandemic.^{41,42}

The COVID-19 pandemic disrupted healthcare systems globally,^{43–46} causing reduced access to specialists, lower hospitalization rates, and fewer diagnoses of, e.g., chronic diseases,^{47,48} sexually transmitted infections,^{49,50} dermatological diseases,⁵⁰ and oncological disorders,^{51–60} as well as a decline in the number of transplantations.^{61–64} Access to primary care facilities was also affected during the pandemic.⁶⁵ Rural areas experienced additional challenges, including limited access to technology, mobility constraints, and fewer healthcare resources.^{66–71} Difficulties in accessing public transport in rural areas may have limited people's mobility in less-developed regions.^{72,73} Fear of contracting COVID-19 also contributed to delayed healthcare-seeking behavior.⁶⁷ In our study, there was no significant difference in overall incidence between the pandemic and post-pandemic periods; however, the incidence in rural areas was significantly lower during the pandemic than during the post-pandemic period, likely for the reasons mentioned above.

Geographic and age-related patterns in herpes zoster incidence

Population-based studies show variable urban–rural patterns. In urban settings, Chan et al. reported a prevalence of 2.03–2.21 per 1,000 persons, highest among individuals aged 70–79.⁷⁴ In contrast, Lionis et al. reported a crude incidence of 1.4 per 1,000 persons in rural Greece.⁷⁵ Li et al. found a markedly higher incidence of HZ in urban than in rural areas (7.65 vs 2.06 per 1,000 persons).⁷⁶ However, other studies, such as Cebrián-Cuenca et al., observed the opposite – rural incidence was significantly higher (8.8 per 1,000 persons vs 3.1 per 1,000 persons in urban populations).⁷⁷ According to the authors, the higher incidence of HZ in rural settings could be biased by higher consultation rates per patient in these areas, owing to the lower number of individuals assigned to each general practitioner outside cities compared with urban areas. In our study, overall incidence did not differ significantly between urban and rural areas, although rural incidence during the pandemic years was significantly lower than during the post-pandemic years.

Multiple recent meta-analyses have identified age as one of the strongest independent predictors of HZ.^{78–82} Internationally, HZ incidence in the general population ranges from 1.2 to 3.4 per 1,000 persons per year,⁸³ increasing to 6–8 per 1,000 persons in adults aged 60 years and to 8–12 per 1,000 persons in those aged 80 years.¹ Similar trends

were reported by Li et al.,⁷⁶ who observed an incidence of 3.43 per 1,000 persons in individuals aged ≥ 50 years and 8.55 per 1,000 persons in those aged ≥ 80 years. In Poland, 70% of hospitalized HZ patients are aged >60 years.⁸⁴

Official Polish data on HZ incidence have been available since 2024 and range from approx. 2.86 to 5.56 per 1,000 persons per year.⁴⁰ The IR observed in our study were slightly lower than those reported in the official Polish data but were consistent with international trends, ranging from 2.14 in 2020 to 1.61 in 2024. Age-related declines in VZV-specific cellular immunity and the presence of comorbidities likely contribute to these outcomes.^{78–80,85–89} In our study, the incidence of HZ per 1,000 persons per year increased with age and was highest in patients aged >80 years in every year except 2020.

Complications of herpes zoster: Incidence and risk factors

Postherpetic neuralgia risk follows a similar pattern. It rarely occurs in individuals younger than 50 years but increases substantially with age, reaching up to 21% in persons aged 80–85 years.^{90,91} In the systematic review by Giannelos et al.,²⁵ PHN incidence varied widely (2.6–46.7%) depending on the population and definitions used. Studies based on primary care databases generally reported lower PHN rates (2.6–13.7%) due to underrecognition of mild and moderate cases.²⁵ Several retrospective analyses support these trends^{91–96}; e.g., Gauthier et al.⁹¹ observed PHN lasting 3 months in 13.7% of patients aged ≥ 50 years, while Alicino et al.⁹² reported PHN in 12.7% of HZ cases (up to 28.57% in those aged ≥ 85 years). Our data similarly demonstrated significantly higher PHN rates in older adults, with 4.47 times the odds of persistent pain in those aged ≥ 71 years.

Herpes zoster ophthalmicus carries a risk of severe ocular complications, including keratitis, uveitis, corneal disease, and stroke.^{97,98} In the general population, ocular involvement occurs in 1.4–15.9% of HZ cases,²⁵ with higher rates in immunocompromised patients (up to 11.4% among patients with solid organ malignancies and 11.3% among those with hematologic malignancies)^{99,100} compared with 4.8% in immunocompetent individuals.¹⁰¹

Our findings align with these reported ranges. Herpes zoster oticus is associated with facial nerve palsy,¹⁰² hearing impairment (24% of adult cases),³⁰ and vertigo.¹⁰³ Although studies on its incidence are limited, estimates range from 1.4% to 1.7%,¹⁰⁴ with values as high as 18%.¹⁰⁵ In our cohort, HZ oticus accounted for 2.28% of all cases, consistent with these values. Disseminated HZ is the rarest but most severe form, occurring in 0.3–8.2% of the general population²⁵ and in 0–0.5% of immunocompetent individuals.^{25,100,101,106} Incidence is higher among patients with hematologic malignancies (0.65–19.3%), among those with solid organ malignancies (0.04–8.5%),^{100,107,108} and among transplant recipients (0–14%).^{100,109} In our study, only 3 cases (0.54%) were identified, likely due to underreporting, as patients

with severe disease or immunocompromise often present directly to hospital units.^{14,110–112} However, this value is consistent with rates reported in other studies.

Sex as a determinant in herpes zoster

Sex differences in HZ risk have been consistently reported. A meta-analysis by Kawai and Yawn found a higher incidence among women (pooled risk ratio (RR) = 1.31; 95% CI: 1.27–1.34).⁸⁰ Li et al. also demonstrated a higher incidence in women in primary care populations (3.95 per 1,000 persons in women vs 2.89 per 1,000 persons in men).⁷⁶ Many studies confirm that women are more likely to develop HZ,^{10,78,81,113–118} though the explanation remains multifactorial. Health-seeking behavior may differ between sexes. Biological mechanisms, such as immune or hormonal modulation, may contribute,^{80,119} and diagnostic biases have also been suggested.^{118,120}

Polish hospital data similarly report a female predominance (58% of HZ hospitalizations).⁸⁴ Our study supports these findings, with women developing HZ more frequently than men; the difference was statistically significant in 2020 (two-proportion test: $f = (2.74, 1.47)$, $p = 0.048$). In that year, the IR per 1,000 persons was 2.74 for women (95% CI: 2.13–3.54) and 1.47 for men (95% CI: 1.02–2.12). However, no significant differences were observed during 2021–2024, likely due to an insufficient sample size; this finding warrants further investigation.^{121,122}

Implications for practice

The strong age-related increase in the risk of HZ and PHN underscores the importance of targeted prevention in older adults. The recent reimbursement of the RZV (Shingrix) in Poland provides an opportunity to reduce disease burden, particularly in high-risk groups such as individuals aged ≥ 60 years, immunocompromised patients, and those living in rural areas with limited health-care access. Strengthening primary care screening and patient education may further mitigate PHN by enabling earlier diagnosis and treatment. Pandemic-related fluctuations in reporting also highlight the need to reinforce surveillance systems to improve trend monitoring and guide vaccination policies.

Limitations of the study

Several limitations must be acknowledged. First, we lacked access to data prior to 2020, as the electronic health record system was fully implemented at the participating clinics only in mid-2019. Consequently, we were unable to compare our findings with data from the pre-pandemic period. Furthermore, although the study involved several clinics, they were all part of the same primary care network, which may limit the generalizability of the findings to other regions or healthcare systems. In addition,

the retrospective design and reliance on electronic health records may have led to diagnostic errors or underreporting, particularly among patients with mild or moderate symptoms. Moreover, due to the factors outlined in the Materials and methods section, the influence of comorbidities and HZ vaccination coverage could not be evaluated, and these aspects require further investigation.

Conclusions

Our study provides an updated analysis of the incidence of HZ and its complications in the primary care population in Poland. We found that age was significantly associated with the incidence of HZ, PHN, and HZO. Moreover, further investigation into the pandemic's impact on rural healthcare is warranted, as we observed differences in HZ incidence between the pandemic and post-pandemic periods in rural areas. It is particularly important for general practitioners to inform patients about the risk of HZ, especially older adults, given that HZ vaccination is available and fully reimbursed for eligible patients in Poland.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.20718265>. The package contains the following files:

Supplementary Table 1. Demographic distribution of HZ cases by year (2020–2024): number of cases (n) and incidence per 1,000 persons per year (IR) with corresponding 95% CIs.

Supplementary Table 2. Results of normality tests for clinical and demographic variables in HZ patients.

Supplementary Fig. 1. Incidence by area of residence in 2020–2024.

Supplementary Fig. 2. Time between first and last visit in the pandemic and post-pandemic periods.

Supplementary Fig. 3. Incidence by area of residence in the pandemic and post-pandemic periods.

Data Availability Statement

The datasets supporting the findings of the current study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.20689609>.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Awareness and acceptance of respiratory syncytial virus (RSV) vaccination among pregnant women in Poland: Results of an internet-based survey

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Abstract

Background. Respiratory syncytial virus (RSV) is a major cause of severe lower respiratory tract infections in infants, particularly among vulnerable groups such as preterm newborns and infants with underlying medical conditions. The approval of the RSVpreF vaccine for use during pregnancy offers a new strategy for reducing RSV-related morbidity in newborns.

Objectives. This study aimed to assess RSV vaccination coverage among pregnant women in Poland, identify factors influencing vaccine hesitancy, and evaluate knowledge regarding the benefits of RSV vaccination.

Materials and methods. A Computer-Assisted Web Interview (CAWI) survey was conducted among 668 respondents in Poland. An anonymous questionnaire consisting of 23 questions was distributed via social media platforms between July 25 and August 1, 2024. Participants were recruited through Facebook groups and Instagram and included women who had recently given birth or were currently pregnant.

Results. Respondents with medical backgrounds more frequently provided correct answers to questions regarding RSV than those without medical training. For example, when asked about methods of treating RSV infection, 109 medical professionals (74.15%) answered correctly, compared with 251 non-medical respondents (48.18%; $p < 0.001$). Individuals with medical backgrounds and those who received information about vaccination from their physician were more likely to receive the RSV vaccine during pregnancy. In addition, women vaccinated against other diseases, such as influenza and pertussis, were more likely to choose RSV vaccination.

Conclusions. Vaccination against RSV during pregnancy is an important strategy for protecting newborns against severe RSV infection and should be promoted as a public health measure. Our findings highlight the need for targeted educational initiatives and public health campaigns to increase awareness and improve vaccination coverage among pregnant women.

Key words: respiratory syncytial virus vaccines, vaccination, pregnant women, vaccine hesitancy, internet-based survey

Highlights

- Pregnant women in Poland show limited awareness and acceptance of RSV vaccination.
- Medical professionals and physician guidance significantly increase RSV vaccine uptake.
- Prior vaccination against influenza or pertussis is associated with greater willingness to receive RSV vaccination.
- Targeted education and public health campaigns are essential to improve RSV vaccination coverage.

Background

Respiratory syncytial virus (RSV) is a seasonal pathogen that places a substantial burden on healthcare systems worldwide. In children, RSV infection can manifest with a broad spectrum of clinical presentations, ranging from mild upper respiratory tract infections to severe lower respiratory tract infections, such as pneumonia and bronchiolitis. These severe manifestations may require hospitalization and can lead to serious complications, including respiratory failure.^{1,2}

Newborns born prematurely and infants with underlying medical conditions are particularly vulnerable to severe RSV infection. High-risk groups include children with chronic lung disease of prematurity (CLDP) or bronchopulmonary dysplasia (BPD), hemodynamically significant congenital heart disease (hsCHD), immunocompromising conditions, and severe neuromuscular disorders.³

For many years, effective methods capable of providing safe and reliable protection for these vulnerable populations were lacking. Despite advances in neonatal and pediatric care, severe RSV infections have continued to pose a significant threat to infant health and survival.

In 2015, the global incidence of RSV-associated acute lower respiratory infection (ALRI) was estimated at 33.1 million cases, resulting in 3.2 million hospitalizations and approx. 59,600 deaths among children younger than 5 years of age. Infants younger than 6 months accounted for a substantial proportion of this disease burden, with an estimated 27,300 deaths worldwide.⁴

A major advance in RSV prevention occurred in August 2023, when the U.S. Food and Drug Administration (FDA) approved the RSVpreF vaccine for use during pregnancy. This approval was followed in the subsequent month by recommendations from the Advisory Committee on Immunization Practices (ACIP) and the Centers for Disease Control and Prevention (CDC). These organizations recommended administration of a single dose of RSVpreF between 32 and 36 completed weeks of gestation to reduce the risk of RSV-associated lower respiratory tract infections in infants.^{5,6}

Vaccination during pregnancy continues to generate discussion and concerns among both patients and healthcare professionals in Poland. According to the 2024 Protective Vaccination Program, recommended vaccinations during pregnancy include influenza and pertussis vaccines.⁷

Objectives

The aim of this study was to assess RSV vaccination coverage among pregnant women, identify factors contributing to vaccine hesitancy, and evaluate knowledge regarding the benefits of RSV vaccination.

Materials and methods

This cross-sectional study was based on an original questionnaire developed by the authors. The survey was distributed via social media platforms between July 25 and August 1, 2024. The questionnaire consisted of 23 items and was designed to ensure anonymous and voluntary participation. The target population included women in the 3rd trimester of pregnancy and women up to 6 months postpartum. Participants were recruited through Facebook groups dedicated to pregnant women, where a link to the questionnaire was posted together with an invitation to participate. In addition, the questionnaire was promoted through Instagram Stories. All participants provided informed consent and were informed of their right to withdraw from the study at any time before submitting the questionnaire. Due to the fully anonymous nature of the survey, individual responses could not be identified or withdrawn after submission.

Qualitative variables were expressed as frequencies and percentages (n, %). Prior to conducting the χ^2 test, the assumption regarding expected cell frequencies was assessed (i.e., expected counts <5 in no more than 20% of cells). If this assumption was met, Pearson's χ^2 test of independence was applied; otherwise, Fisher's exact test was used for 2 × 2 contingency tables (Statistica v. 13.3.721.1; StatSoft, Tulsa, USA). The level of statistical significance was set at $p < 0.05$. When multiple comparisons were performed, a Bonferroni correction was applied by dividing 0.05 by the number of comparisons. During analyses examining associations between responses and specific factors, the response category "maybe" was excluded because it accounted for only 1% of responses. As a measure of effect size in contingency table analyses, Cramér's V coefficient was calculated, where values close to 0 indicate little or no association and values close to 1 indicate a strong association between the analyzed variables. The study protocol was submitted to the Bioethics

Committee of Wrocław Medical University (Poland), which determined that formal ethical approval was not required (opinion No. Kb_kanc 235/2024). The study was assessed as compliant with ethical standards for scientific research, including the principles outlined in the Declaration of Helsinki, and was not considered to violate the rights of the surveyed participants.

Results

A survey was conducted among 668 women between July 25 and August 1, 2024. The respondents ranged in age from 18 to 42 years. Table 1 presents the demographic characteristics of the study population. The primary objective

of the study was to assess women’s knowledge of RSV infection and awareness of its potential consequences. Knowledge was evaluated using a set of author-developed questions, and respondents were categorized as either medical professionals or non-medical respondents. Statistically significant differences between the 2 groups were observed for only 3 questions: “What are the methods of treating RSV infection?”, “Is RSV vaccination recommended during pregnancy?” and “Do you know how RSV vaccines work?”. Detailed results are presented in Table 2. We also assessed respiratory syncytial virus (RSV) vaccination uptake among women working in medical professions and those without medical education (Table 3). Women with medical backgrounds were significantly more likely to receive the RSV vaccine during pregnancy than non-medical respondents. In addition, we examined how frequently physicians informed patients about the availability of RSV vaccination and whether women who received this information from their physician were more likely to choose vaccination than those who did not receive such information (Table 4). Finally, we evaluated the association between uptake of other recommended vaccinations during pregnancy, such as influenza and pertussis vaccines, and RSV vaccination uptake. Detailed results are presented in Table 5.

Discussion

Respiratory syncytial virus (RSV) is the leading cause of acute lower respiratory tract infections and a major contributor to infant mortality, particularly among children younger than 6 months of age in low- and middle-income

Table 1. Sociodemographic characteristics of the respondents

Variable	Category	n (%)
Age [years]	18–26	38 (5.69)
	27–35	468 (70.06)
	over 35	162 (24.25)
Number of inhabitants	village	122 (18.26)
	city up to 50,000 inhabitants	95 (14.22)
	city between 50,000 and 500,000 inhabitants	164 (24.55)
	city over 500,000 inhabitants	287 (42.96)
Education	secondary	71 (10.63)
	technical	2 (0.30)
	higher education	595 (89.07)
Medical profession	yes	147 (22.01)
	no	521 (77.99)

Table 2. Comparison of knowledge regarding respiratory syncytial virus (RSV) between medical and non-medical respondents, n (%)

Question	Correct answer	Are you in the medical profession?			Statistics		
		yes	no	FE/ χ^2	df	p-value	V
What is RSV?	yes	145 (98.64)	511 (98.08)	FE	–	0.921	0.02
	no	2 (1.36)	10 (1.92)				
What are the symptoms of RSV infection?	yes	140 (95.24)	475 (91.17)	$\chi^2 = 2.07$	1	0.150	0.06
	no	7 (4.76)	46 (8.83)				
What age groups are most at risk of complications after RSV infection?	yes	86 (58.50)	334 (64.11)	$\chi^2 = 1.31$	1	0.252	0.05
	no	61 (41.50)	187 (35.89)				
What are the methods of treating RSV infection?	yes	109 (74.15)	251 (48.18)	$\chi^2 = 30.09$	1	<0.001	0.22
	no	38 (25.85)	270 (51.82)				
Do you know what causes RSV infection in newborns and infants?	yes	127 (86.39)	394 (75.62)	$\chi^2 = 7.13$	1	0.008	0.11
	no	20 (13.61)	127 (24.38)				
Is the RSV vaccination recommended in pregnancy?	yes	118 (80.27)	318 (61.04)	$\chi^2 = 18.71$	1	<0.001	0.17
	no	29 (19.73)	203 (38.96)				
Do you know how RSV vaccines work?	yes	117 (79.59)	267 (51.25)	$\chi^2 = 37.69$	1	<0.001	0.24
	no	30 (20.41)	254 (48.75)				

FE – Fisher’s exact test (no test statistic reported); df – degrees of freedom; V – Cramér’s V coefficient for contingency tables. Due to multiple comparisons, a Bonferroni correction was applied. The significance threshold was adjusted from $\alpha = 0.05$ to $\alpha = 0.006$ (0.05/8).

Table 3. Comparison of RSV vaccination uptake between medics and non-medics, n (%) (Pearson's χ^2 test of independence)

Question	Answer	Are you in the medical profession?		χ^2	p-value	V
		yes	no			
Have you been vaccinated against RSV during pregnancy?	yes	37 (25.17)	67 (13.01)	12.77	<0.001	0.14
	no	110 (74.83)	448 (86.99)			

V – Cramér's V coefficient for contingency tables; $\alpha = 0.05$.

Table 4. Comparison of respiratory syncytial virus (RSV) vaccination rates in pregnant patients informed about RSV vaccination during pregnancy by their physician and those not informed about RSV vaccination by their physician, n (%) (Pearson's χ^2 test of independence)

Question	Answer	Did your physician inform you about the possibility of RSV vaccination?*		χ^2	p-value	V
		yes	no			
Have you been vaccinated against RSV during pregnancy?	yes	99 (44.39)	5 (1.14)	208.95	<0.001	0.56
	no	124 (55.61)	434 (98.86)			

*Where do you obtain information about RSV vaccination? (multiple-choice question): Yes – respondents who indicated “physician” as a source of information, either as the sole response or among multiple selected responses; No – respondents who did not indicate “physician” as a source of information; V – Cramér's V coefficient for contingency tables; $\alpha = 0.05$.

Table 5. Association between respiratory syncytial virus (RSV) vaccination uptake and receipt of other recommended vaccinations during pregnancy (Pearson's χ^2 test of independence)

Question	Answer	Were you vaccinated against other diseases during pregnancy?		χ^2	p-value	V
		yes	no			
Have you been vaccinated against RSV during pregnancy?	yes	100 (23.47)	4 (1.69)	54.40	<0.001	0.29
	no	326 (76.53)	232 (98.31)			

V – Cramér's V coefficient for contingency tables; $\alpha = 0.05$.

countries.^{8,9} Despite the fact that vaccines have saved thousands, if not millions, of lives, vaccine hesitancy and opposition to vaccination remain significant public health challenges. Anti-vaccination movements have existed since the introduction of vaccines and continue to influence public attitudes toward immunization. In Poland, despite the existence of mandatory vaccination programs, the number of vaccine exemptions nearly doubled over the past 5 years, increasing from 48,600 in 2019 to 87,300 in 2023.¹⁰ This trend highlights a growing public health concern that requires attention in the coming years. Similar patterns have also been observed in other countries, including the USA, India, and neighboring Ukraine.¹¹

The introduction of the RSV vaccine has attracted considerable attention, particularly with regard to concerns about its safety and effectiveness. The most commonly reported local and systemic adverse reactions include pain at the injection site, headache, myalgia, and nausea. Nevertheless, attention has also focused on other safety signals observed during clinical trials. Concerns have been raised regarding potential risks such as preterm birth and hypertensive disorders of pregnancy; however, these findings did not reach statistical significance. These observations prompted the U.S. Food and Drug Administration (FDA) to issue a warning regarding the potential risk of preterm birth, particularly among individuals vaccinated before 32 weeks of gestation. In addition, a higher proportion

of infants born to vaccinated mothers were reported to have low birth weight ($\leq 2,500$ g) and neonatal jaundice compared with infants born to mothers who received placebo. Given these observations, ongoing research and post-marketing surveillance remain essential to ensure that the benefits of RSV vaccination continue to outweigh any potential risks. It is equally important that healthcare professionals remain well informed in order to provide evidence-based recommendations to their patients.¹²

Vaccination during pregnancy to confer passive immunity to the newborn has been practiced for more than a century. Historical references to maternal smallpox vaccination date back to the 1870s. Maternal immunoglobulin G (IgG) antibodies generated in response to natural infection or active immunization during pregnancy cross the placenta and enter the fetal circulation, providing newborns with protection during the first months of life.^{13,14} Given these well-established benefits, the persistently low uptake of maternal vaccination and the concerns frequently expressed by pregnant women remain a source of concern for healthcare professionals.

In recent years, maternal vaccination has become a standard component of preventive healthcare and has been incorporated into numerous national immunization programs. Recommendations regarding specific vaccines vary among countries due to differences in epidemiological, social, and healthcare-related factors. For example, pertussis

vaccination during pregnancy has been implemented in many low- and middle-income countries, whereas influenza vaccination has been incorporated into immunization schedules in most high-income countries and several middle-income countries.¹⁵

Maternal vaccination promotes the transplacental transfer of high concentrations of maternal antibodies, providing infants with immediate protection at birth and throughout the first months of life.^{16–18} This strategy is currently used to protect infants against tetanus, pertussis, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and influenza.^{19,20} Such an approach is particularly important in low- and middle-income countries, where the burden of RSV-associated lower respiratory tract infections is greatest.⁸

One study evaluating RSV vaccination during pregnancy in Argentina demonstrated that vaccination of 342,110 pregnant women with RSVpreF prevented an estimated 3,915 RSV-related hospitalizations, 6,399 RSV-related emergency department visits, and 67 RSV-related deaths.²¹

Following recommendations issued by the CDC regarding vaccine safety, numerous vaccinations are recommended during pregnancy, with particular emphasis on vaccines that have been demonstrated to be safe and effective for both mother and child. In Poland, recommendations developed by the National Institute of Public Health – National Research Institute in collaboration with the Polish Vaccinology Society include vaccination against influenza, pertussis, COVID-19, and, more recently, RSV.^{22,23}

Given that leading medical organizations consider vaccination during pregnancy to be both safe and effective, it is concerning that a substantial proportion of eligible women still choose not to receive recommended vaccines.

According to a study conducted by Wilcox et al. in 2019, the most common reason for declining vaccination was concern about potential adverse effects on the child.²⁴ Consequently, the findings of our study are particularly relevant, as they suggest that insufficient knowledge about vaccination may contribute to fear and uncertainty among patients. Wilcox et al. also demonstrated that healthcare providers often lacked confidence in communicating information about vaccination. In contrast, our study showed that healthcare professionals possessed more accurate and comprehensive knowledge regarding RSV vaccination than respondents without a medical background.²⁴

Additional factors influencing vaccination uptake during pregnancy include ethnicity, access to vaccination through the recommending healthcare provider, and parity. According to a study by McAuslane et al., pertussis vaccination uptake was significantly lower among women who had given birth more than 3 times (48.1%; 95% confidence interval (95% CI): 37.0–59.4) than among those with up to 3 previous births (63.0%; 95% CI: 58.3–67.5).²⁵

Licata et al. reported similarly noteworthy findings, demonstrating that 71.8% of healthcare professionals informed

women about influenza vaccination during pregnancy, while 68.8% actively recommended influenza vaccination during pregnancy. Their study population included obstetricians-gynecologists, midwives, and primary care physicians involved in the care of pregnant women. These findings are consistent with the pattern observed in our study regarding RSV vaccination uptake. Although an RSV vaccination rate of 25% among pregnant healthcare professionals may appear relatively low, it was significantly higher than that observed among women without a healthcare background. Furthermore, the findings reported by Licata et al., demonstrating that good knowledge and positive attitudes toward vaccination were the strongest predictors of recommending influenza vaccination during pregnancy, are consistent with our observations regarding RSV vaccination uptake.²⁶

Kherfan and Sallam reported findings similar to ours, demonstrating that among 315 respondents, only 67.6% had heard of RSV before participating in their study. Furthermore, respondents declared that if the RSV vaccine were safe, effective, and available free of charge, 70.2% would be willing to receive it, whereas 15.2% would refuse and 14.6% would remain hesitant. These findings are encouraging and suggest considerable potential for increasing RSV vaccine uptake, particularly when compared with the vaccination rates observed in our study.²⁷

According to available evidence, Poland is not the only country considering the permanent inclusion of RSV vaccination in national immunization recommendations. Papagiannis et al. conducted a study in response to the potential introduction of new RSV vaccination guidelines by the National Vaccines Committee, aiming to assess knowledge, attitudes, and anticipated vaccination practices among healthcare professionals in Central Greece. A particularly encouraging finding was that healthcare professionals demonstrated a high level of knowledge regarding RSV, which was strongly associated with positive attitudes toward RSV vaccination and a greater willingness to recommend it. Similar observations can be drawn from our study, in which medical education was associated with a higher likelihood of RSV vaccination during pregnancy. These findings emphasize the importance of educational interventions targeting both healthcare professionals and patients to improve vaccination uptake.²⁸

As reported by Nowacka et al., one of the most common reasons for declining COVID-19 vaccination was insufficient knowledge about the vaccine. Lessons learned from the introduction of SARS-CoV-2 vaccines may therefore be highly relevant for the implementation of RSV vaccination programs. Women with higher educational attainment (odds ratio (OR) = 2.26, $p < 0.001$), those who received positive information about vaccination (OR = 2.74, $p = 0.017$), and those informed about the potential complications of COVID-19 during pregnancy (OR = 2.60, $p < 0.001$) were more likely to accept vaccination. Accordingly, the authors emphasized the importance of appropriate education,

training, and promotion of vaccination. Our findings support this conclusion, as women who received information about RSV vaccination from healthcare professionals were more likely to accept vaccination than those who relied on other sources of information or had not received any information.²⁹

It is important to note that, from April 1, 2025, RSV vaccination has been reimbursed in Poland. This policy change may substantially increase vaccine uptake. At the time our survey was conducted, the cost of RSV vaccination was approx. 800–1,000 PLN. The inclusion of RSV vaccination in the reimbursement program was specified in the Announcement of the Minister of Health of March 19, 2025, concerning the list of reimbursed medicines, foods for special medical purposes, and medical devices, effective from April 1, 2025.³⁰

Limitations of the study

The primary limitation of this study is that data collection relied on social media platforms and online survey distribution. However, experience gained during the COVID-19 pandemic has demonstrated that online surveys can serve as an effective, safe, and accessible tool for this type of research, enabling the collection of data from a large number of participants. We acknowledge that our sample was disproportionately composed of women with higher educational attainment and those working in healthcare professions. We also recognize that these individuals may hold more established views on vaccination, which could have influenced the findings. Consequently, these factors may have affected the representativeness of the sample and, therefore, the results of the analysis.

Conclusions

RSV vaccination during pregnancy represents an important strategy for protecting newborns against RSV infection and its severe consequences. However, awareness of RSV vaccination and vaccine uptake among pregnant women in Poland remain suboptimal. The findings of this study may support public health authorities and other stakeholders in developing future RSV vaccination policies and strategies. Furthermore, these results may help guide the design of targeted educational initiatives aimed at increasing awareness of RSV and RSV vaccination among both healthcare professionals and pregnant women.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.17349531>. The package contains the following files:

Supplementary Table 1. General characteristics of the study respondents.

Supplementary Table 2. Comparison of RSV-related knowledge between medical and non-medical respondents.

Supplementary Table 3. Comparison of RSV vaccination rates between medical and non-medical respondents.

Supplementary Table 4. Association between physician-provided information about RSV vaccination and RSV vaccination uptake.

Supplementary Table 5. Association between vaccination against other infectious diseases during pregnancy and RSV vaccination uptake.

Data Availability Statement

The datasets supporting the findings of the current study are openly available in Zenodo at <https://doi.org/10.5281/zenodo.17349673>.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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HDL-related inflammatory biomarkers are associated with sleep disorders: Evidence from a cross-sectional NHANES study

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;

D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Sleep disorders are common and heterogeneous conditions associated with systemic inflammation. They can impair quality of life and increase disease burden. Currently, studies investigating the association between sleep disorders and high-density lipoprotein (HDL)-related inflammatory biomarkers are lacking.

Objectives. This study aimed to explore the associations between HDL-related inflammatory biomarkers and sleep disorders.

Materials and methods. This study utilized data from the National Health and Nutrition Examination Survey (NHANES; 2005–2014). Multivariable logistic regression models were applied to explore the associations between HDL-related inflammatory biomarkers, including platelet-to-HDL ratio (PHR), lymphocyte-to-HDL ratio (LHR), neutrophil-to-HDL ratio (NHR), and monocyte-to-HDL ratio (MHR), and the prevalence of sleep disorders and sleep duration. Subgroup analyses were conducted to investigate potential interactions. In addition, the nonlinear relationship between these inflammatory biomarkers and the likelihood of sleep disorders was assessed using restricted cubic spline (RCS) models. Finally, the robustness of the observed associations was examined through sensitivity analyses.

Results. A total of 23,045 individuals were included. In the fully adjusted models, higher MHR (odds ratio (OR) = 1.33; 95% confidence interval (95% CI): 1.11–1.60), NHR (OR = 1.25; 95% CI: 1.04–1.49), LHR (OR = 1.24; 95% CI: 1.05–1.48), and PHR (OR = 1.18; 95% CI: 1.00–1.39) were positively associated with an increased prevalence of sleep disorders. The RCS analysis revealed linear relationships between these inflammatory biomarkers and the likelihood of sleep disorders (all *p* for nonlinearity >0.05). Subgroup analyses indicated that sex and marital status significantly interacted with all 4 indices (all *p* for interaction <0.05). Compared with individuals with the recommended sleep duration (7–9 h/day), those with short (<7 h/day) and long (>9 h/day) sleep durations had elevated NHR levels.

Conclusions. This study revealed a linear association between HDL-related inflammatory biomarkers and sleep disorders. Moreover, sex and marital status modified the associations of all 4 indices with sleep disorders. In addition, these inflammatory biomarkers were associated with sleep duration.

Key words: sleep disorders, high-density lipoproteins, inflammation mediators, biomarkers, National Health and Nutrition Examination Survey (NHANES)

Highlights

- This study systematically investigated the association between HDL-related inflammatory biomarkers and sleep disorders.
- Our study observed distinct associations between HDL-related inflammatory indices and sleep duration.
- A linear association between HDL-related inflammatory biomarkers and sleep disorders was observed. Sex and marital status interacted with all 4 indices.
- These findings may offer new perspectives on the inflammatory mechanisms underlying sleep disorders and support the potential utility of HDL-related inflammatory indices.

Background

Sleep is a vital physiological process essential for maintaining normal bodily functions and brain activity. It plays a key role in restoring physical and neurological systems, enhancing cognitive performance, and promoting mental wellbeing.¹ Sleep disorders have become a major global public health issue, with a steadily increasing prevalence affecting approx. 30% of the global population.² According to the International Classification of Sleep Disorders, 3rd Edition (ICSD-3), sleep disorders are classified into 7 categories: insomnia, sleep-related breathing disorders, circadian rhythm sleep-wake disorders, parasomnias, central disorders of hypersomnolence, sleep-related movement disorders, and other sleep disorders.³ Currently, the underlying mechanisms of sleep disorders remain incompletely understood, and no definitive diagnostic biomarkers are available. Moreover, the diagnosis of sleep disorders often relies on complex and costly polysomnography.⁴ Notably, chronic sleep disorders are closely associated with a range of serious health outcomes, including obesity, hypertension, type 2 diabetes, cardiovascular disease (CVD), and increased all-cause mortality.⁵ Given the substantial disease burden associated with sleep disorders, identifying modifiable biomarkers is of significant value for developing preventive strategies against sleep disorders.

Inflammation plays a central role in the pathophysiology of sleep disorders. It can affect the central nervous system through neural signaling, humoral mediators, and transport across the blood–brain barrier (BBB), thereby modulating neurotransmitter systems and disrupting sleep.⁶ High-density lipoprotein (HDL) plays a pivotal role in lipid metabolism by transporting lipids from peripheral tissues back to the liver, a process known as reverse cholesterol transport.⁷ Beyond its well-known cardiovascular protective effects, increasing evidence supports the anti-inflammatory, antioxidant, and immunomodulatory functions of HDL.⁸ The complexity of HDL arises from its composition of multiple subunits, each with unique protein and lipid components that play key regulatory roles in both the innate and adaptive immune systems.⁹ On the one hand, HDL exerts anti-inflammatory effects by inhibiting the release of inflammatory cytokines, neutralizing

endotoxins, modulating vascular endothelial cell function, and regulating immune cell activity.¹⁰ On the other hand, systemic inflammation can impair HDL function by reducing its antioxidant capacity and cholesterol efflux efficiency and may even promote a pro-inflammatory phenotype.¹¹

Notably, inflammation can reach the central nervous system through direct neural pathways, humoral mediators, and transport across the BBB via active mechanisms and cellular routes, thereby modulating neurotransmitter systems and ultimately disrupting sleep.⁶ Previous studies have reported associations between sleep disorders and various inflammatory markers (including HDL and C-reactive protein (CRP)), as well as metabolic indicators (such as HbA1c (glycated hemoglobin), blood glucose, and insulin).¹² However, research specifically focusing on HDL-related inflammatory biomarkers in relation to sleep disorders remains lacking. It is hypothesized that composite indices reflecting the overall inflammatory and immunoregulatory functions of HDL may better reveal the underlying inflammatory pathology of sleep disorders than individual blood cell counts or lipid parameters and could potentially serve as novel diagnostic or risk assessment markers.

In recent years, a series of HDL-related inflammatory indices derived from routine blood tests, such as the lymphocyte-to-HDL cholesterol ratio (LHR), monocyte-to-HDL cholesterol ratio (MHR), neutrophil-to-HDL cholesterol ratio (NHR), and platelet-to-HDL cholesterol ratio (PHR), have emerged as novel biomarkers. Due to their accessibility and low cost, these indices have been extensively studied and have demonstrated significant value in risk prediction across various conditions, including CVD, metabolic syndrome, and periodontitis.^{13–16} However, whether these HDL-associated inflammatory indices are associated with sleep disorders remains unclear.

Objectives

We hypothesized that higher levels of HDL-related inflammatory biomarkers (MHR, NHR, LHR, and PHR) would be positively associated with sleep disorders and abnormal sleep duration. To test this hypothesis, we aimed to investigate these associations using data from

the National Health and Nutrition Examination Survey (NHANES). Since these indices are derived from routine blood counts and lipid profiles, confirming their association with sleep disorders could provide clinicians with a simple, cost-effective tool for the early identification of high-risk individuals. This would facilitate targeted interventions, offering a practical biomarker-based approach for screening and prevention, with potentially significant implications for public health and quality of life.

Materials and methods

Data source and study population

The NHANES, conducted by the National Center for Health Statistics (NCHS) of the U.S. Centers for Disease Control and Prevention (CDC), aims to assess the health and nutritional status of adults and children in the USA. The survey protocol was approved by the National Center for Health Statistics (NCHS) Research Ethics Review Board (ERB) under its continuous protocol for NHANES (ERB Approval Protocols #2005-06 and #2011-17). All participants provided written informed consent.

As illustrated in Fig. 1, data from 5 consecutive NHANES cycles (2005–2014), including U.S. individuals aged 20 years and older, were collected. To ensure comprehensiveness and validity, individuals were excluded if they: 1) lacked assessment data on sleep disorders or sleep duration; 2) had incomplete data on platelet, neutrophil, lymphocyte, monocyte counts, or HDL cholesterol (HDL-C); or 3) had missing covariate data, including age, sex, race, education level, marital status, smoking status, alcohol consumption, diabetes, hypertension, CVD, or body mass index (BMI).

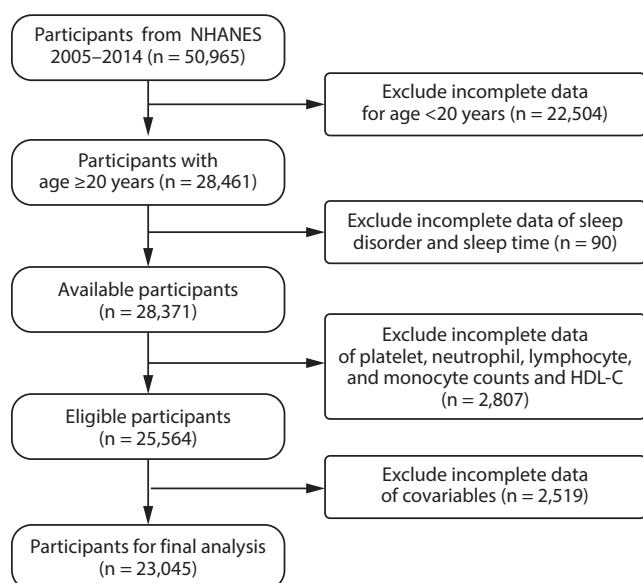


Fig. 1. Diagram of the participant enrollment process

HDL-C – high-density-lipoprotein cholesterol.

Exposure variables

Exposure variables, including monocyte, lymphocyte, neutrophil, and platelet counts, as well as HDL-C levels, were obtained from the whole blood cell count database of NHANES. The MHR was calculated as monocyte/HDL-C, NHR as neutrophil/HDL-C, PHR as platelet/HDL-C, and LHR as lymphocyte/HDL-C. Given the skewed distributions of these indices, log transformation was applied when the 4 biomarkers were analyzed as continuous variables.¹⁶

Outcome variables

Sleep disorders were determined based on responses to NHANES item SLQ060: “Have you ever been told by a doctor or other health professional that you have a sleep disorder?” Responses included “Don’t know,” “No,” “Yes,” and “Refused.” Individuals with missing responses or those answering “Don’t know” or “Refused” were excluded. Sleep duration was evaluated using the question: “How many hours of sleep do you usually get?” Following the National Sleep Foundation recommendations, sleep duration was categorized as short (<7 h/day), recommended (7–9 h/day), or long (>9 h/day).¹⁷

Covariates

Demographic and lifestyle factors were collected, including age, sex, race, education level, marital status, smoking status, alcohol consumption, diabetes, hypertension, CVD, and BMI. Age was categorized as <40, 40–60, and >60 years. Race was categorized as non-Hispanic Black, Hispanic, Mexican American, non-Hispanic White, or other. Marital status was grouped as widowed, married, separated, divorced, cohabiting, or never married. Education level was categorized as below high school, high school diploma, or above. Drinkers were defined as individuals who consumed more than 12 alcoholic drinks per year. Smokers were defined as individuals who had smoked more than 100 cigarettes in their lifetime. Body mass index was classified as ≤ 25 kg/m² or > 25 kg/m². Alcohol intake frequency was categorized as 1–5 times/month, 5–10 times/month, ≥ 10 times/month, never, or unknown. Smoking status was classified as current, former, or never smoker. The diagnoses of diabetes and hypertension were based on responses to the questionnaire item: “Has a doctor ever diagnosed you with diabetes or hypertension?” Cardiovascular disease included angina, stroke, myocardial infarction, heart failure, and coronary heart disease.

Statistical analyses

Sample weights were applied in accordance with the NHANES 2005–2014 guidelines (<https://wwwn.cdc.gov/nchs/nhanes/tutorials/weighting.aspx>). Considering

the 5 consecutive 2-year cycles, sample weights were adjusted by dividing the 2-year examination center weights (WTMEC2YR) by 5 to ensure nationally representative estimates. For all continuous variables subjected to intergroup comparisons, we tested for normality within each study group separately. For variables that were normally distributed in all groups, results are presented as mean $\pm$ standard deviation (mean $\pm$ SD). An independent samples t-test was used for comparisons if the assumption of homogeneity of variances was met; otherwise, Welch's t test was applied. For continuous variables that were not normally distributed in any of the study groups, results are presented as median (interquartile range (IQR)), and the Mann–Whitney U test was used for intergroup comparisons. The results of the normality and homogeneity tests are provided in Supplementary Table 1. Categorical variables were expressed as numbers and percentages (n (%)) and were compared between groups using the χ^2 test. We reviewed all contingency tables used in the analysis and verified that the minimum expected frequency in each cell exceeded 5, thereby satisfying the assumptions of Pearson's χ^2 test. Detailed results are provided in Supplementary Table 2. Potential multicollinearity among covariates was evaluated using variance inflation factors (VIFs), and all covariates included in the models had VIF values below 10, indicating no significant multicollinearity in the present study (Supplementary Table 3). Additionally, the linearity assumption between continuous predictors and the logit of the outcome was assessed using the Box–Tidwell test. Detailed results are provided in Supplementary Table 4. Weighted multivariable logistic regression models were applied to examine the associations between HDL-related inflammatory indices and the presence of sleep disorders. Weighted multivariable logistic regression models were further used to investigate the associations between these biomarkers and sleep duration, categorized as short (<7 h/day), recommended (7–9 h/day), or long (>9 h/day). Model 1 was unadjusted; Model 2 was adjusted for key demographic variables, including sex, age, and race; Model 3 was further adjusted for education level, marital status, smoking status, alcohol consumption, diabetes, hypertension, CVD, and BMI. Restricted cubic splines (RCS) with 3 knots placed at the 25th, 50th, and 75th percentiles were used to investigate the nonlinear association between the HDL-related inflammatory markers and sleep disorders. Subgroup analyses were conducted to examine whether the associations between HDL-related inflammatory indices and sleep disorders varied across different population characteristics, and likelihood ratio tests were performed to assess potential interactions between the HDL-related inflammatory indices and these characteristics. Sensitivity analyses were also conducted to verify the robustness of the results. First, unweighted data were used for repeated analyses. Second, propensity score matching (PSM) using nearest-neighbor

matching at a 1 : 3 ratio was performed to balance group differences. Following matching, the primary analysis was repeated. The relevant code can be found in Supplementary File 1. Third, outliers were addressed using the winsorization method, and regression analyses were subsequently performed based on the processed data. All statistical analyses were conducted using R software v. 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria), and a two-sided $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of study population

Baseline characteristics of the included individuals were compared according to the presence or absence of sleep disorders (Table 1). A total of 23,045 individuals were included, of whom 48.93% were male. Among participants in the sleep-disorder group, 52.64% were male, 51.73% were aged 40–60 years, 75.93% were non-Hispanic White, 84.82% had a high-school education or above, 58.27% were married, 84.13% had a BMI > 25 kg/m², 38.10% consumed alcohol 1–5 times per month, and 44.06% had never smoked. The prevalence of hypertension among individuals with sleep disorders was 52.07%. The results showed that individuals with sleep disorders had significantly higher monocyte and neutrophil counts, along with lower HDL-C levels, resulting in elevated MHR, LHR, NHR, and PHR values (all $p < 0.05$).

Associations between HDL-related inflammatory indices and sleep disorders

The HDL-related inflammatory biomarkers were treated as continuous variables in the regression models. In Model 3 (fully adjusted), positive associations were observed between sleep disorders and log(MHR) (odds ratio (OR) = 1.30, 95% confidence interval (95% CI): 1.10–1.52), log(NHR) (OR = 1.21, 95% CI: 1.05–1.38), log(LHR) (OR = 1.18, 95% CI: 1.03–1.36), and log(PHR) (OR = 1.20, 95% CI: 1.02–1.43) (Table 2). Subsequently, these 4 indices were categorized into tertiles for further analysis. In Model 3, compared with the lowest tertile (T1), the highest tertile (T3) was significantly associated with a greater likelihood of sleep disorders (MHR (OR = 1.33, 95% CI: 1.11–1.60), NHR (OR = 1.25, 95% CI: 1.04–1.49), LHR (OR = 1.24, 95% CI: 1.05–1.48), and PHR (OR = 1.18, 95% CI: 1.00–1.39)) (Table 2).

In addition, RCS analyses were performed to further investigate the nonlinear relationships between the 4 indices and sleep disorders. As illustrated in Fig. 2 the results suggested linear associations between all 4 indices and the likelihood of sleep disorders (p for nonlinearity >0.05).

Table 1. Weighted characteristics of the study population (n = 23,045)

Characteristic		n	Overall	Non-sleep disorder n (91.4%)	Sleep disorder n (8.6%)	p-value
Gender	female	23,045	11,687 (51.07%)	10,765 (51.43%)	922 (47.36%)	0.007
	male		11,358 (48.93%)	10,348 (48.57%)	1,010 (52.64%)	
Age	<40	23,045	7,874 (36.31%)	7,497 (37.79%)	377 (20.74%)	<0.001
	40–60		8,045 (40.16%)	7,171 (39.06%)	874 (51.73%)	
	>60		7,126 (23.53%)	6,445 (23.15%)	681 (27.53%)	
Race	Mexican American	23,045	3,659 (8.23%)	3,463 (8.56%)	196 (4.73%)	<0.001
	Non-Hispanic Black		4,680 (10.46%)	4,285 (10.51%)	395 (9.97%)	
	Non-Hispanic White		10,754 (70.04%)	9,706 (69.48%)	1,048 (75.93%)	
	other Hispanic		2,050 (4.99%)	1,863 (5.02%)	187 (4.72%)	
	other race		1,902 (6.27%)	1,796 (6.42%)	106 (4.65%)	
Education	below high school	23,045	5,880 (16.97%)	5,449 (17.13%)	431 (15.18%)	0.042
	high school or above		17,165 (83.03%)	15,664 (82.87%)	1,501 (84.82%)	
Marital status	divorced	23,045	2,487 (10.42%)	2,192 (10.03%)	295 (14.54%)	<0.001
	living with partner		1,775 (7.62%)	1,642 (7.71%)	133 (6.66%)	
	married		12,116 (56.55%)	11,086 (56.39%)	1,030 (58.27%)	
	never married		4,066 (17.38%)	3,822 (17.93%)	244 (11.64%)	
	separated		742 (2.28%)	662 (2.20%)	80 (3.06%)	
	widowed		1,859 (5.75%)	1,709 (5.74%)	150 (5.82%)	
BMI [kg/m ²]	≤25	23,045	6,805 (30.96%)	6,519 (32.39%)	286 (15.87%)	<0.001
	>25		16,240 (69.04%)	14,594 (67.61%)	1,646 (84.13%)	
Alcohol consumption	1–5 drinks/month	23,045	8,695 (40.00%)	7,993 (40.17%)	702 (38.10%)	<0.001
	10+ drinks/month		3,337 (17.55%)	3,113 (17.89%)	224 (13.99%)	
	5–10 drinks/month		1,838 (9.65%)	1,717 (9.82%)	121 (7.79%)	
	non-drinker		6,475 (22.98%)	5,939 (22.92%)	536 (23.69%)	
	unknown		2,700 (9.82%)	2,351 (9.20%)	349 (16.42%)	
Smoker	current smoker	23,045	4,879 (21.03%)	4,407 (20.74%)	472 (24.14%)	<0.001
	former smoker		5,695 (24.84%)	5,087 (24.18%)	608 (31.80%)	
	never smoker		12,471 (54.13%)	11,619 (55.08%)	852 (44.06%)	
Hypertension	no	23,045	14,879 (68.31%)	14,043 (70.24%)	836 (47.93%)	<0.001
	yes		8,166 (31.69%)	7,070 (29.76%)	1,096 (52.07%)	
Diabetes	no	23,045	20,320 (91.28%)	18,833 (92.26%)	1,487 (80.94%)	<0.001
	yes		2,725 (8.72%)	2,280 (7.74%)	445 (19.06%)	
CVD	no	23,045	20,609 (91.65%)	19,116 (92.68%)	1,493 (80.71%)	<0.001
	yes		2,436 (8.35%)	1,997 (7.32%)	439 (19.29%)	
HDL [mg/dL]		23,045	51.00 (42.00, 62.00)	51.00 (42.00, 62.00)	47.00 (39.00, 57.00)	<0.001
HDL [mmol/L]		23,045	1.32 (1.09, 1.60)	1.32 (1.09, 1.60)	1.22 (1.01, 1.47)	<0.001
Lymphocyte count [1000 cells/uL]		23,045	2.00 (1.60, 2.50)	2.00 (1.60, 2.50)	2.00 (1.60, 2.50)	0.200
Neutrophil count [1000 cells/uL]		23,045	4.00 (3.20, 5.20)	4.00 (3.10, 5.10)	4.20 (3.30, 5.40)	<0.001
Monocyte count [1000 cells/uL]		23,045	0.50 (0.40, 0.70)	0.50 (0.40, 0.60)	0.60 (0.40, 0.70)	<0.001
Platelet count [1000 cells/uL]		23,045	244.00 (207.00, 288.00)	244.00 (207.00, 288.00)	243.00 (205.00, 286.00)	0.200
PHR		23,045	185.54 (143.33, 239.46)	184.29 (142.50, 237.82)	197.64 (156.03, 253.23)	<0.001
MHR		23,045	0.40 (0.29, 0.55)	0.39 (0.29, 0.54)	0.46 (0.33, 0.63)	<0.001
LHR		23,045	1.53 (1.10, 2.09)	1.52 (1.10, 2.08)	1.67 (1.21, 2.27)	<0.001
NHR		23,045	3.10 (2.19, 4.31)	3.06 (2.16, 4.26)	3.55 (2.51, 4.91)	<0.001

Median (interquartile range (IQR)) was used to represent variables with a non-normal distribution, and differences between groups were compared using the Wilcoxon test. Categorical data were expressed as percentages, and differences between groups were compared using the χ^2 test. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; PHR – platelet-to-high-density lipoprotein ratio; LHR – lymphocyte-to-high-density lipoprotein ratio; MHR – monocyte-to-high-density lipoprotein ratio; NHR – neutrophil-to-high-density lipoprotein ratio.

Table 2. Relationship between HDL-related inflammation index and sleep disorder

Participants		Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
MHR	log (MHR)	1.82 (1.58–2.09)	<0.001	1.83 (1.58–2.12)	<0.001	1.30 (1.10–1.52)	0.002
	T1	ref.		ref.		ref.	
	T2	1.30 (1.11–1.53)	0.002	1.32 (1.12–1.55)	0.001	1.08 (0.91–1.28)	0.400
	T3	1.92 (1.63–2.26)	<0.001	1.93 (1.63–2.30)	<0.001	1.33 (1.11–1.60)	0.003
	p for trend	<0.001		<0.001		0.005	
NHR	log (NHR)	1.70 (1.51–1.93)	<0.001	1.80 (1.59–2.03)	<0.001	1.21 (1.05–1.38)	0.007
	T1	ref.		ref.		ref.	
	T2	1.32 (1.11–1.57)	0.002	1.35 (1.13–1.61)	0.001	1.08 (0.90–1.30)	0.400
	T3	1.86 (1.61–2.16)	<0.001	1.96 (1.69–2.27)	<0.001	1.25 (1.04–1.49)	0.018
	p for trend	<0.001		<0.001		0.040	
LHR	log (LHR)	1.50 (1.33–1.70)	<0.001	1.70 (1.49–1.95)	<0.001	1.18 (1.03–1.36)	0.018
	T1	ref.		ref.		ref.	
	T2	1.21 (1.05–1.38)	0.009	1.33 (1.14–1.54)	<0.001	1.06 (0.92–1.23)	0.400
	T3	1.62 (1.40–1.87)	<0.001	1.90 (1.61–2.24)	<0.001	1.24 (1.05–1.48)	0.013
	p for trend	<0.001		<0.001		0.032	
PHR	log (PHR)	1.65 (1.41–1.93)	<0.001	1.88 (1.60–2.22)	<0.001	1.20 (1.02–1.43)	0.032
	T1	ref.		ref.		ref.	
	T2	1.35 (1.16–1.56)	<0.001	1.42 (1.21–1.65)	<0.001	1.16 (0.99–1.35)	0.068
	T3	1.58 (1.36–1.83)	<0.001	1.77 (1.52–2.07)	<0.001	1.18 (1.00–1.39)	0.048
	p for trend	<0.001		<0.001		0.086	

Model 1: unadjusted for any covariates. Model 2: adjusted for age, sex, and race. Model 3: adjusted for age, sex, race, education level, marital status, BMI, smoking status, alcohol consumption, cardiovascular disease, hypertension, and diabetes. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; PHR – platelet-to-high-density lipoprotein ratio; LHR – lymphocyte-to-high-density lipoprotein ratio; MHR – monocyte-to-high-density lipoprotein ratio; NHR – neutrophil-to-high-density lipoprotein ratio; OR – odds ratio; 95% CI – 95% confidence interval; p for trend: derived by treating ordinal tertile groups (T1–T3) as a continuous variable in regression models, tested using the Wald test.

Subgroup analysis

To assess associations across different population subgroups, subgroup analyses were performed (Fig. 3–6). Significant interactions with sex and marital status were observed for all 4 indices. Smoking status interacted with MHR (p for interaction = 0.033) and NHR (p for interaction < 0.001), but not with LHR (p for interaction = 0.088) or PHR (p for interaction = 0.093). All 4 indices were associated with sleep disorders among males and married participants. Among never smokers, MHR, NHR, and LHR, but not PHR, showed significant associations.

Associations between HDL-related inflammatory indices and sleep duration

Regression models were used to examine the associations between HDL-related inflammatory markers (as continuous variables) and sleep duration. In Model 3, compared with the recommended sleep duration (7–9 h/day), log(NHR) was positively associated with long sleep duration (>9 h/day; OR = 1.39, 95% CI: 1.17–1.66) and short sleep duration (<7 h/day; OR = 1.06, 95% CI: 1.00–1.13). Log(MHR) was positively associated with long sleep

duration (OR = 1.33, 95% CI: 1.10–1.61), whereas log(LHR) was positively associated with short sleep duration (OR = 1.10, 95% CI: 1.03–1.17). As illustrated in Table 3, there was no evidence of significant associations between log(PHR) and either short or long sleep duration.

Sensitivity analysis

The robustness of the associations was examined using 3 sensitivity analyses. First, the primary analysis was repeated using unweighted data. In Model 3, log(MHR) (OR = 1.29, 95% CI: 1.13–1.47), log(NHR) (OR = 1.27, 95% CI: 1.12–1.45), and log(LHR) (OR = 1.29, 95% CI: 1.14–1.47) remained positively associated with the prevalence of sleep disorders, whereas no significant association was observed for log(PHR) (Supplementary Table 5). Second, PSM was employed to control for potential confounding (Fig. 7). After matching, differences in covariates, including age, sex, marital status, race, BMI, smoking status, alcohol consumption, education level, diabetes, hypertension, and CVD, were successfully balanced between groups (Supplementary Table 6). Post-matching analyses indicated that log(MHR) (OR = 1.42, 95% CI: 1.16–1.73) and log(NHR) (OR = 1.25, 95% CI: 1.03–1.52) remained significantly

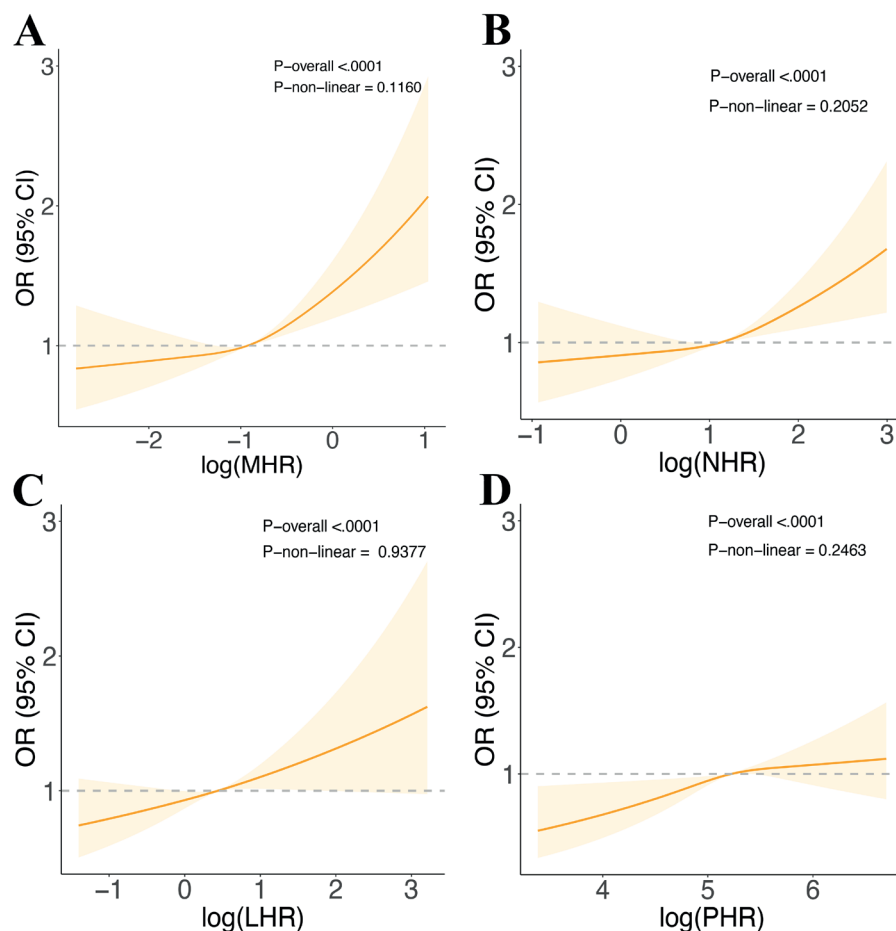


Fig. 2. Association between HDL-related inflammatory indices and sleep disorders. A. Association between MHR and sleep disorders; B. Association between NHR and sleep disorders; C. Association between LHR and sleep disorders; D. Association between PHR and sleep disorders

HDL – high-density lipoprotein; MHR – monocyte-to-HDL ratio; NHR – neutrophil-to-HDL ratio; PHR – platelet-to-HDL ratio; LHR – lymphocyte-to-HDL ratio.

associated with a greater likelihood of sleep disorders (Supplementary Table 7). Third, outliers of the exposure variables ($\log(\text{MHR})$, $\log(\text{NHR})$, $\log(\text{LHR})$, and $\log(\text{PHR})$) were handled using the winsorization method, followed by regression analyses. In Model 3, positive associations were observed between sleep disorders and $\log(\text{MHR})$ (OR = 1.30, 95% CI: 1.10–1.52), $\log(\text{NHR})$ (OR = 1.21, 95% CI: 1.05–1.38), $\log(\text{LHR})$ (OR = 1.18, 95% CI: 1.03–1.36), and $\log(\text{PHR})$ (OR = 1.20, 95% CI: 1.02–1.43) (Supplementary Table 8).

Discussion

This study, based on NHANES data, examined the associations between HDL-related inflammatory biomarkers and sleep disorders. The results indicated that NHR, LHR, MHR, and PHR were positively associated with the likelihood of sleep disorders. Furthermore, RCS analyses confirmed linear relationships between these indices and the prevalence of sleep disorders. Subgroup analyses indicated that sex and marital status significantly modified the associations of all 4 indices with sleep disorders, whereas smoking status significantly modified the associations of MHR and NHR only. This study also found that elevated MHR was significantly associated with long sleep duration, whereas higher NHR was significantly associated with both long

and short sleep durations. In addition, elevated LHR was significantly associated with short sleep duration.

Previous studies have mainly focused on the relationship between sleep disorders and abnormalities in lipid metabolism. Multiple cross-sectional studies revealed a characteristic lipid pattern in individuals with circadian rhythm disorders and sleep-related breathing disorders. This pattern is characterized by higher levels of triglycerides, total cholesterol, and low-density lipoprotein, together with lower HDL levels.^{18,19} The underlying mechanism may be associated with impaired anti-inflammatory functions of HDL.^{20,21} In contrast to previous research, this study not only confirmed reduced HDL levels in individuals with sleep disorders but also revealed a novel pattern of inflammatory imbalance involving platelets, neutrophils, and monocytes. By introducing multidimensional inflammatory ratios (MHR, NHR, LHR, and PHR), this study systematically elucidated the lipid-immune interaction network implicated in the pathophysiology of sleep disorders. This finding goes beyond the conventional framework based on individual lipid markers and may provide a valuable perspective for identifying novel clinical biomarkers.

This study suggests that HDL dysfunction, rather than a mere reduction in HDL concentration, might contribute to the development of sleep disorders through impaired anti-inflammatory activity. Existing evidence supports the role of HDL-related inflammatory indices as promising

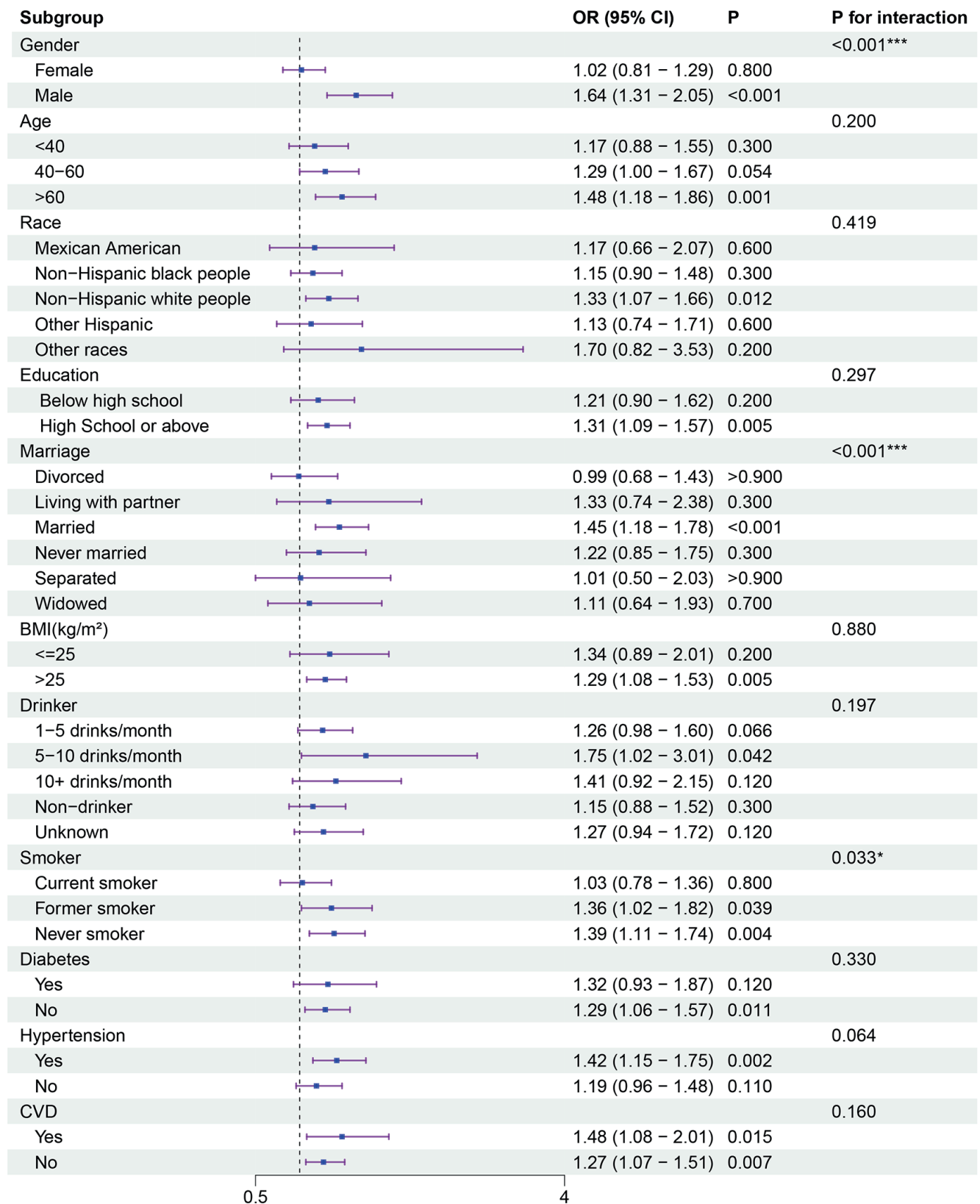


Fig. 3. Subgroup analysis of the association between MHR and sleep disorders. Odds ratios (ORs) and 95% confidence intervals (95% CIs) are shown. Interaction p-values were calculated using likelihood ratio tests

* $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; MHR – monocyte-to-HDL ratio.

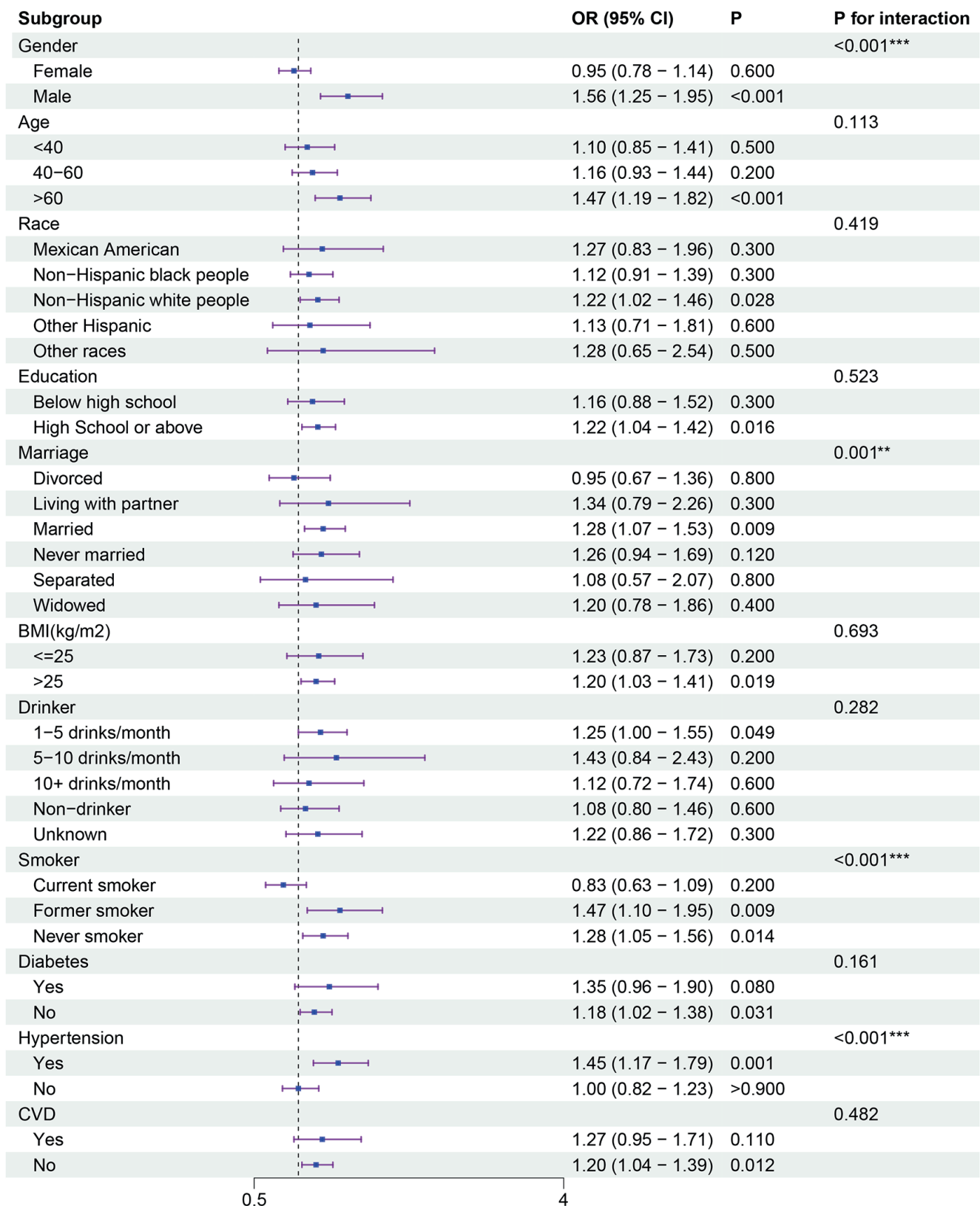


Fig. 4. Subgroup analysis of the association between NHR and sleep disorders. Odds ratios (ORs) and 95% confidence intervals (95% CIs) are shown. Interaction p-values were calculated using likelihood ratio tests

*p < 0.050; **p < 0.010; ***p < 0.001. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; NHR – neutrophil-to-HDL ratio.

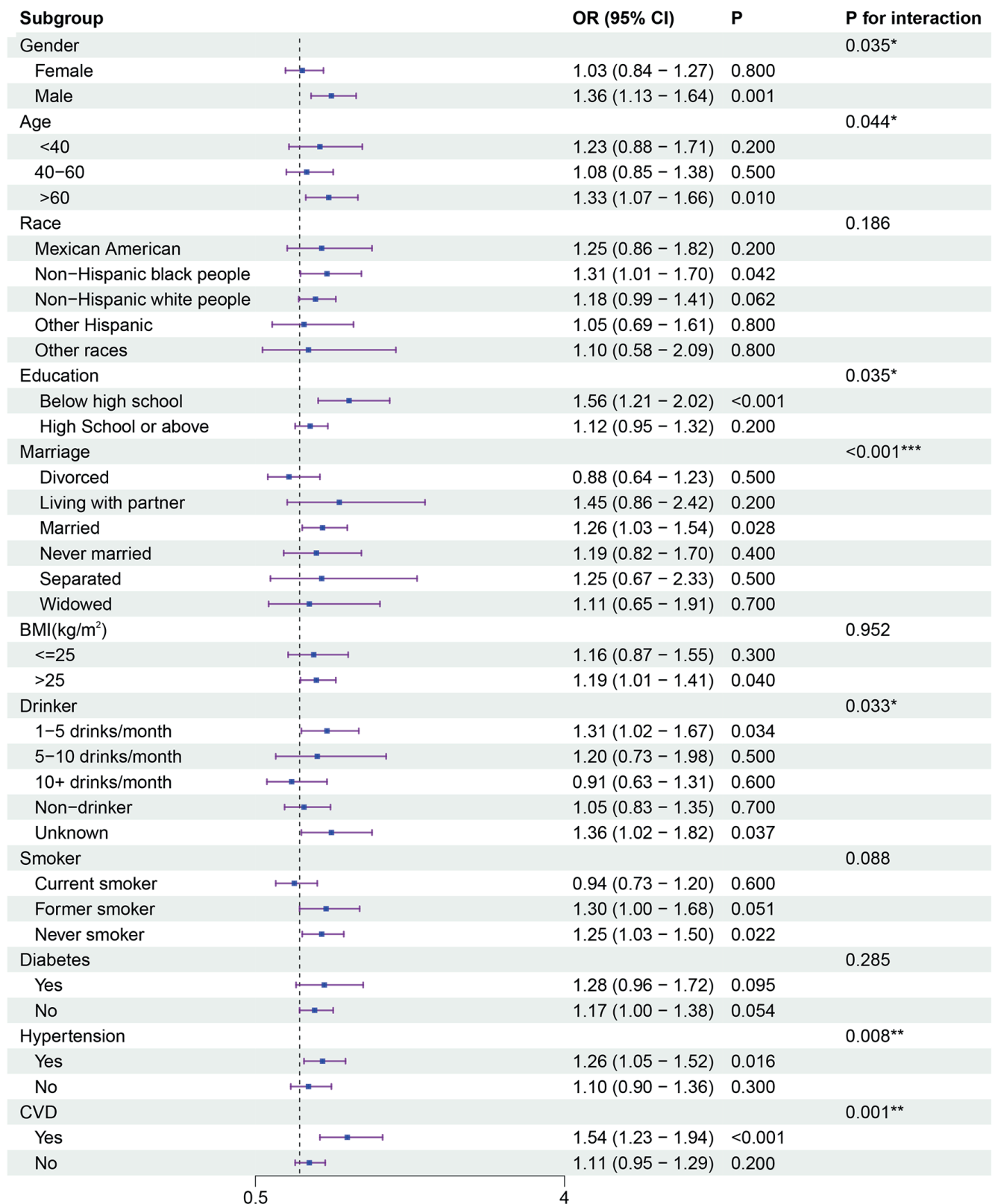


Fig. 5. Subgroup analysis of the association between LHR and sleep disorders. Odds ratios (ORs) and 95% confidence intervals (95% CIs) are shown. Interaction p-values were calculated using likelihood ratio tests

* $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; LHR – lymphocyte-to-HDL ratio.

biomarkers for sleep disorders. Meng et al. demonstrated that MHR was positively associated with the severity of obstructive sleep apnea (OSA).²² Their findings indicate that

MHR may serve as an independent biomarker for the diagnosis of OSA and may help improve clinical assessment and guide targeted intervention strategies.²² These findings

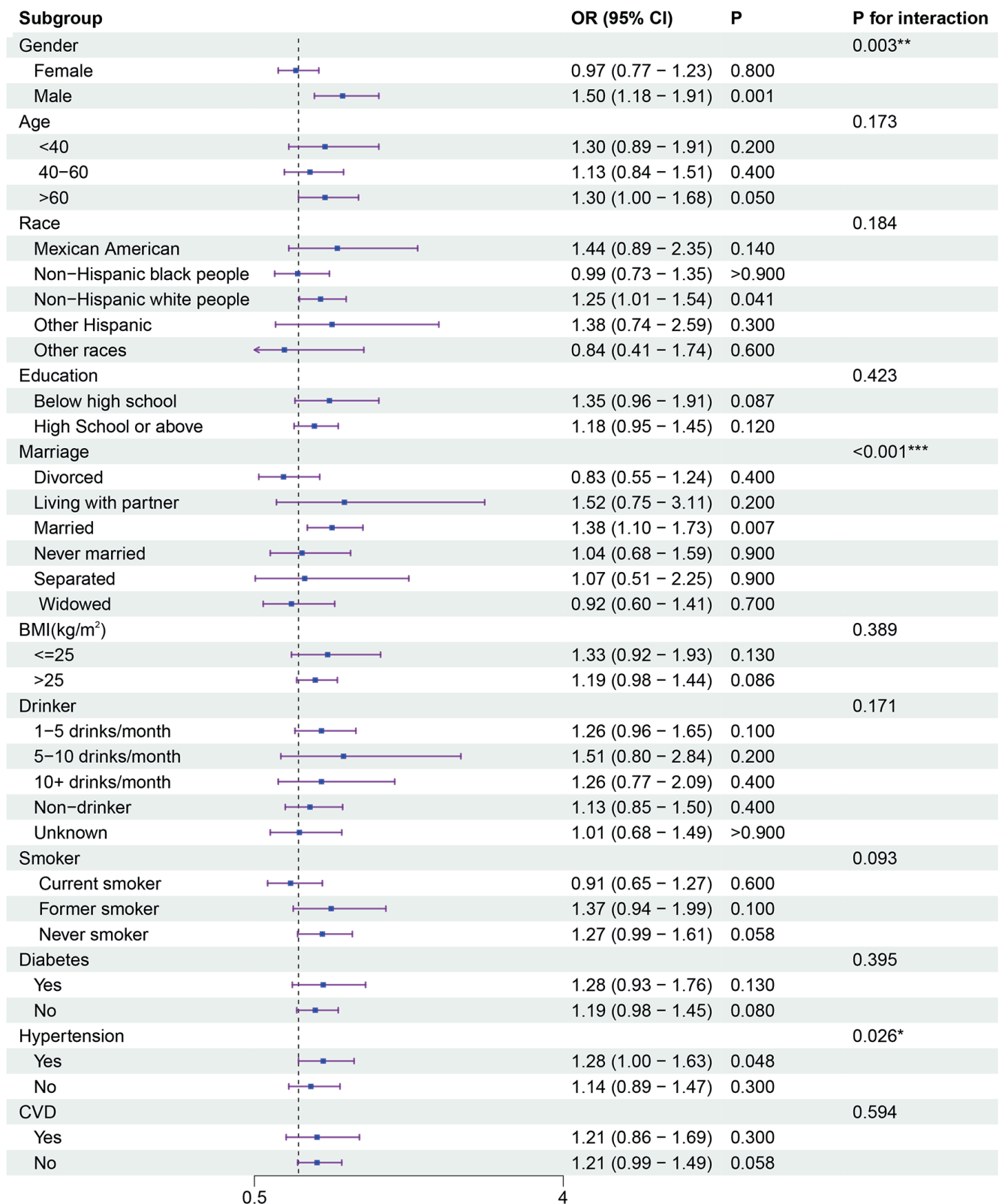


Fig. 6. Subgroup analysis of the association between PHR and sleep disorders. Odds ratios (ORs) and 95% confidence intervals (95% CIs) are shown. Interaction p-values were calculated using likelihood ratio tests

*p < 0.050; **p < 0.010; ***p < 0.001. BMI – body mass index; CVD – cardiovascular disease; HDL – high-density lipoprotein; PHR – platelet-to-HDL ratio.

are consistent with our results. Using a larger representative sample of American adults, our study demonstrated that MHR, NHR, and LHR were significantly associated

with the development and progression of sleep disorders. These indices have also been proposed as early predictors of various diseases, including atherosclerosis, CVD, type 2

Table 3. Relationship between HDL-related inflammation index and sleep time

Participants	Sleep duration	Model 1		Model 2		Model 3	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Log(MHR)	7–9 h/day	ref.		ref.		ref.	
	<7 h/day	1.09 (1.03–1.15)	0.002	1.22 (1.15–1.29)	<0.001	1.06 (0.99–1.13)	0.092
	>9 h/day	1.41 (1.19–1.66)	<0.001	1.55 (1.29–1.85)	<0.001	1.33 (1.10–1.61)	0.003
Log(NHR)	7–9 h/day	ref.		ref.		ref.	
	<7 h/day	1.09 (1.03–1.14)	0.001	1.06 (1.04–1.07)	<0.001	1.06 (1.00–1.13)	0.042
	>9 h/day	1.42 (1.21–1.66)	<0.001	1.09 (1.06–1.12)	<0.001	1.39 (1.17–1.66)	<0.001
Log(LHR)	7–9 h/day	ref.		ref.		ref.	
	<7 h/day	1.27 (1.20–1.34)	<0.001	1.26 (1.19–1.34)	<0.001	1.10 (1.03–1.17)	0.003
	>9 h/day	1.12 (0.95–1.32)	0.185	1.27 (1.07–1.50)	0.005	1.11 (0.93–1.32)	0.247
Log(PHR)	7–9 h/day	ref.		ref.		ref.	
	<7 h/day	1.22 (1.14–1.31)	<0.001	1.24 (1.16–1.33)	<0.001	1.07 (0.99–1.15)	0.088
	>9 h/day	1.08 (0.88–1.33)	0.447	1.26 (1.02–1.55)	0.030	1.10 (0.88–1.37)	0.392

Model 1: unadjusted for any covariates. Model 2: adjusted for age, sex, and race. Model 3: adjusted for age, sex, race, education level, marital status, BMI, smoking status, alcohol consumption, cardiovascular disease, hypertension, and diabetes. BMI – body mass index; PHR – platelet-to-high-density lipoprotein ratio; LHR – lymphocyte-to-high-density lipoprotein ratio; MHR – monocyte-to-high-density lipoprotein ratio; NHR – neutrophil-to-high-density lipoprotein ratio; OR – odds ratio; 95% CI – 95% confidence interval.

diabetes, and first-onset psychiatric disorders, all of which frequently co-occur with sleep disorders.^{23–25}

The pathogenesis of sleep disorders is closely associated with immune status and systemic inflammation. During inflammation, activated immune cells (such as lymphocytes and neutrophils) regulate immune responses by releasing pro- and anti-inflammatory mediators.²⁶ Neutrophils, as key effectors in the early stages of inflammation, release chemokines to recruit lymphocytes and monocytes, thereby triggering adaptive immune responses.²⁷ Through HDL-mediated inflammatory regulation, monocytes constitute a dynamic network involved in oxidative stress responses, signal transduction, and lipid homeostasis.²⁸ Interactions between HDL and immune cells, including neutrophils, monocytes, and lymphocytes, can impair the anti-inflammatory effects of HDL.^{29–31} Inflammation reduces the antioxidant and anti-inflammatory activities of HDL and increases lipid hydroperoxides, pro-inflammatory proteins, and oxidized high-density lipoprotein (HDL_{ox}), ultimately compromising its anti-inflammatory functions.^{32–34} The inflammatory microenvironment may induce immune dysfunction and disrupt neuroendocrine regulation, contributing to the onset and progression of neuropsychiatric disorders.³⁵ Peripheral inflammatory cytokines cross the BBB, initiate neuroinflammation, and facilitate the development of mental illness.³⁶ Persistent neuroinflammation leads to structural damage, such as neuronal atrophy and excessive synaptic pruning, and disrupts hypothalamic–pituitary–adrenal (HPA) axis regulation. Consequently, neuroendocrine dysfunction may impair circadian rhythm regulation, thereby contributing to the development of sleep disorders.²⁷

Subgroup analyses indicated significant interactions with sex and marital status for all 4 indices, whereas

smoking status interacted significantly only with MHR and NHR. Among never smokers, MHR, NHR, and LHR, but not PHR, were significantly associated with sleep disorders. This finding may reflect 2 intersecting biological mechanisms: 1) stronger associations in males may be related to the testosterone–estrogen balance. Animal studies have reported that testosterone can enhance neutrophil chemotaxis by upregulating the TLR4/NF- κ B signaling pathway.³⁷ Estrogen can inhibit interleukin (IL)-6 secretion from monocytes, potentially providing a protective effect in females.³⁸ These results suggest that sex-specific cut-off values should be established for inflammatory indices when used as biomarkers. 2) Stronger associations in married individuals may reflect the impact of marriage-related stress on immune homeostasis. Chronic social stress can promote the differentiation of hematopoietic stem cells in the bone marrow toward pro-inflammatory monocytes and enhance platelet aggregation via HPA axis activation.³⁹ Notably, this “social stress–inflammation amplification” effect may be particularly pronounced in ostensibly healthy individuals (such as non-smokers). This might be due to the absence of the anti-inflammatory effects of nicotine on $\alpha 7$ nicotinic acetylcholine receptors.⁴⁰ Nonetheless, the anti-inflammatory effects of nicotine represent a double-edged sword. Its mechanism involves complex neuroimmune interactions. Although it shows therapeutic potential under certain pathological conditions, its clinical application remains limited. Advances in chemical modification or targeted drug delivery technologies are needed to overcome this barrier.

Sleep duration can modulate the immune system, and growing evidence suggests a relationship between sleep duration and inflammatory biomarkers.^{41,42} Prior research has indicated that blood cell-based inflammatory markers,

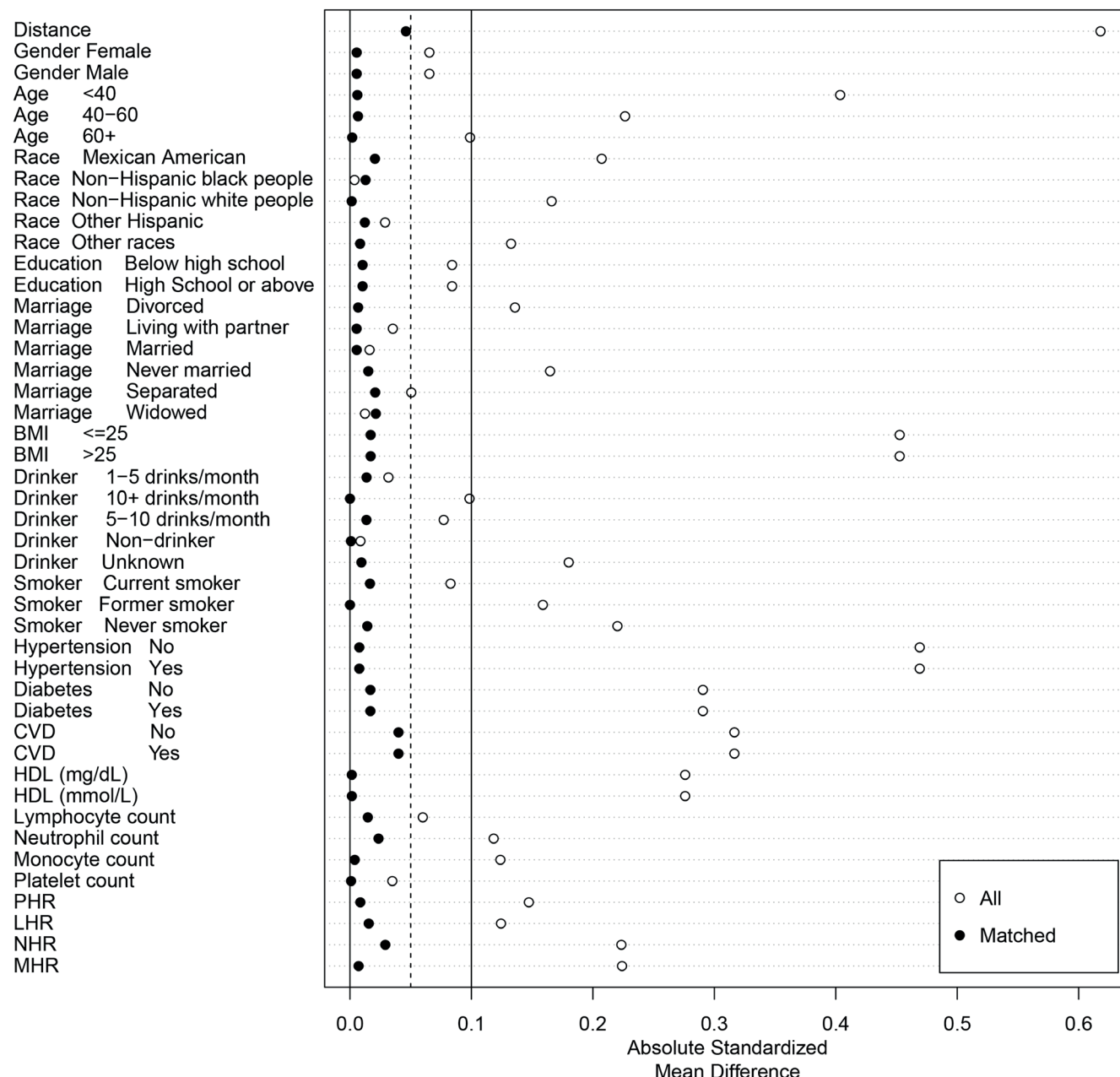


Fig. 7. Standardized mean differences before and after propensity score matching (PSM)

such as the systemic immune-inflammation index, white blood cell count, neutrophil count, and the neutrophil-to-lymphocyte ratio, may mediate the relationship between sleep duration and cognitive decline.⁴³ The present study observed distinct association patterns between MHR, NHR, and LHR (which reflect the dynamic activity of the immune–metabolic axis) and sleep duration, suggesting that this axis may play heterogeneous roles in the regulation of sleep-related inflammation.⁴⁴ Specifically, elevated MHR was associated with longer sleep duration, whereas higher NHR was related to both short and long sleep durations. In contrast, elevated LHR was associated with shorter sleep duration.

The association between prolonged sleep duration and elevated inflammatory markers may reflect underlying

pathological conditions, such as sleep fragmentation, OSA, or the early asymptomatic stages of neurodegenerative diseases.^{45,46} Notably, prolonged but poor-quality sleep, especially when driven by sleep fragmentation or OSA, can induce intermittent hypoxia and tissue stress. These stressors may activate the HPA axis and sympathetic nervous system (SNS), leading to increased release of chemokines (such as MCP-1/CCL2) and enhanced mobilization of monocytes from the bone marrow into peripheral tissues.⁴⁷ Once activated, monocytes can differentiate into macrophages and release pro-inflammatory cytokines (such as IL-6 or tumor necrosis factor alpha (TNF- α)), contributing to a chronic low-grade inflammatory state.⁴⁸ This pathway may be a key source of elevated inflammatory biomarkers among long sleepers.

In addition to monocyte-driven inflammation, the elevated NHR values observed in both short and long sleep durations underscore the high sensitivity of neutrophils, which are rapid responders of innate immunity, to sleep disorders. Short sleep duration is associated with heightened sympathetic activity and hyperactivation of the HPA axis. Repeated activation of this system may trigger pro-inflammatory responses and elevate circulating levels of inflammatory markers.⁶ Short sleep duration can strongly activate the SNS and trigger catecholamine release, thereby rapidly mobilizing neutrophil reserves from the bone marrow into the circulation.⁴⁹ In contrast, prolonged sleep, especially with coexisting OSA or sleep fragmentation, may lead to intermittent hypoxia and tissue stress, potentially activating inflammatory signaling pathways such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B). This, in turn, may stimulate the production of granulocyte colony-stimulating factor and promote neutrophil proliferation and activation.⁵⁰ Activated neutrophils can release pro-inflammatory effectors, including myeloperoxidase and neutrophil extracellular traps. Therefore, neutrophils may serve as a key cellular bridge linking sleep duration and systemic inflammation.

The association between elevated LHR and short sleep duration may reflect complex interactions between adaptive immune dysregulation and stress resulting from sleep deprivation. Short sleep can chronically activate the HPA axis and SNS, exerting bidirectional effects on lymphocyte subsets: 1) potentially suppressing the function of certain anti-inflammatory regulatory T cells⁵¹; 2) promoting the activation of, or altering the distribution of, pro-inflammatory T-cell subsets such as Th1 and Th17⁵²; and 3) impairing the cytotoxic activity of natural killer (NK) cells, although changes in their numbers may be complex.⁵³ Alterations in the proportions and functions of these lymphocyte subsets may collectively disrupt immune homeostasis and promote a pro-inflammatory environment.⁵⁴ As a composite marker, an elevated LHR may reflect these complex dynamic changes in lymphocytes. Although these pathways offer plausible biological explanations, causal relationships require experimental validation through sleep intervention studies combined with immunophenotyping and animal models investigating the time-dependent migration of immune cells.

Using the NHANES database, this study enrolled a nationally representative adult population ($n = 23,045$). The rigorous sampling design and standardized data collection procedures substantially enhanced the generalizability of the findings. Compared with previous small-scale or single-center studies, the large sample size in our study not only improved statistical power but also minimized the risk of bias due to population heterogeneity. This was the first study to systematically examine the association between HDL-related inflammatory biomarkers and sleep disorders. Our study extended beyond

the traditional focus on HDL-C concentration and may offer new insights into the impact of lipid dysfunction on the pathophysiology of sleep disorders. By integrating multiple analytical approaches, including regression, RCS, subgroup, and interaction analyses, this study uncovered complex association patterns (such as threshold effects and population specificity) between HDL-related inflammatory biomarkers and sleep disorders, thereby avoiding the limitations of conclusions based on a single analytical method. Robust statistical methods were employed throughout the study. Sensitivity analyses and PSM were applied to balance intergroup confounding and validate the stability of the associations involving MHR, NHR, LHR, and PHR. To minimize the impact of residual confounding factors, a multivariable logistic regression model was employed, adjusting for demographic characteristics, lifestyle factors, and metabolic disorders.

Limitations of the study

Nonetheless, some limitations should be acknowledged. First, although statistical analyses identified associations between HDL-related inflammatory markers and sleep disorders, the cross-sectional design precluded the inference of temporal or causal relationships. Future prospective cohort studies or Mendelian randomization studies are required to explore these causal relationships. Second, the definition of sleep disorders in this study primarily relied on self-reported questionnaires, lacking support from objective diagnostic measures. This approach has certain limitations, such as misclassification due to subjective bias, inaccurate perception of sleep status, and difficulty distinguishing sleep disorder subtypes. These factors may affect the internal and external validity, as well as the generalizability, of the findings. To obtain more reliable and comprehensive assessments and enhance clinical applicability, future research should incorporate objective measurements, such as polysomnography for diagnostic evaluation and actigraphy for long-term monitoring, to validate and complement self-reported data. Third, a major limitation of this study is the performance of multiple statistical hypothesis tests. While this approach facilitated the exploration of multiple aspects of our research question, it also increased the risk of type I errors. Given the exploratory nature of the study, we did not apply formal correction for multiple comparisons. Consequently, we strongly urge readers to exercise caution when interpreting the statistically significant findings reported in this study and to recognize that some of the observed associations may represent false-positive findings arising by chance. Future confirmatory studies utilizing independent samples with predefined primary hypotheses and appropriate correction methods are warranted to verify the reliability of these findings.

Conclusions

This study identified a linear association between HDL-related inflammatory biomarkers and sleep disorders. Significant interactions with sex and marital status were observed. Moreover, these indices were also associated with sleep duration. These findings may provide new insights into the inflammatory mechanisms underlying sleep disorders and highlight the potential role of HDL-related inflammatory indices in preventive medicine, particularly in informing early intervention strategies.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.17737165>. The package contains the following files:

Supplementary Table 1. Results of normality and homogeneity of variance tests for continuous variables.

Supplementary Table 2. χ^2 test expected frequencies.

Supplementary Table 3. Statistical assessment of collinearity in regression analysis.

Supplementary Table 4. Box–Tidwell test results for log odds linearity.

Supplementary Table 5. Association between HDL-related inflammation index and sleep disorders (unadjusted model).

Supplementary Table 6. Baseline characteristics of participants after PSM ($n = 7,728$).

Supplementary Table 7. Association between HDL-related inflammation index and sleep disorder after PSM.

Supplementary Table 8. Association between HDL-associated inflammatory markers and sleep disorders after outlier treatment by winsorization.

Supplementary File 1. RCS and PSM analysis code.

Data Availability Statement

Data sharing does not apply to this article, as all data are already included in the manuscript and its supplementary files.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable

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E-learning preferences of rural general practitioners: A descriptive educational needs assessment. Results of a cross-sectional survey distributed through UK and European rural GP networks

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Abstract

Background. Rural general practitioners (GPs) encounter unique challenges in accessing continuing professional development (CPD). E-learning offers a potential solution by overcoming geographic and time constraints, particularly when the content is relevant to the rural primary care context.

Objectives. To identify rural GPs' preferences regarding the format, duration, and content of e-learning and to assess their current engagement with and awareness of CPD specific to rural primary care.

Materials and methods. A single cross-sectional online survey was distributed through 2 recruitment channels: the Royal College of General Practitioners (RCGP) in the UK and the European Rural and Isolated Practitioners Association (EURIPA), a pan-European network of the World Organization of Family Doctors (WONCA). A total of 157 RCGP members responded to the survey, with an unknown response rate; 30 of 94 active EURIPA members responded after the survey was distributed through its newsletter and Google Group. The survey assessed e-learning engagement, preferred formats and durations, and priority topics. Descriptive statistics were used to summarize the responses.

Results. Five of the 67 survey participants who answered the question on whether they used any rural primary care-relevant e-learning responded positively (8%). Preferred formats for rural primary care-relevant e-learning included interactive e-modules, webinars, online lectures, and podcasts; the preferred duration was 30–40 min. Priority topics included emergency care, mental health and addiction, multimorbidity and complexity, managing risk and uncertainty, frailty, and telemedicine.

Conclusions. Rural GPs favor short, interactive, context-specific e-learning. These findings can guide the design of pan-European rural primary care modules. Future studies should evaluate educational effectiveness and implementation feasibility.

Key words: rural health, general practitioners, primary healthcare, continuing education, telemedicine

Highlights

- Rural GPs prefer short, interactive e-learning for continuing education.
- Emergency care and dealing with medical complexity, risk and uncertainty are top rural primary care learning priorities.
- Findings support development of pan-European rural primary care-relevant e-learning.

Background

In 2023, approx. 21% of Europe's population lived in rural areas.¹ Primary care, specifically general practitioners (GPs), plays a central role in delivering healthcare to rural populations, often serving as the first point of contact for patients and coordinating ongoing care. Rural GPs and the communities they serve face unique challenges that differ from those of their urban counterparts.² They often provide health and urgent care in resource-poor healthcare environments, have limited access to specialist services compared with urban practitioners, necessitating a broader scope of practice and high skill levels to deal with risk and uncertainty, and care for an expanding rural elderly population with multimorbidity.^{1,3,4} In addition to clinical demands, rural practitioners often deal with professional isolation, compounded by challenges in recruiting and retaining healthcare professionals.^{2,5}

Despite increasing recognition of the distinct nature of rural primary care, there remains a limited understanding of how this shapes the specific educational needs of rural GPs. Rural healthcare professionals frequently encounter additional barriers to fulfilling continuing professional development (CPD) requirements, including time constraints, passive learning formats, and insufficient institutional support.⁶ Existing educational models often reflect urban-centric assumptions, which may not be applicable or effective in rural environments. This underlines the need for context-sensitive, flexible, and practitioner-informed approaches to CPD that address the realities of rural primary care delivery.

Continuing professional development, often facilitated through continuing medical education (CME), is a formal requirement for GPs in many countries, including those practicing in rural settings.⁷ Evidence suggests that peer interaction among rural practitioners can also mitigate stress and reduce the risk of burnout, contributing positively to professional wellbeing and potentially increasing retention.⁸ The European Academy of Teachers in General Practice and Family Medicine (EURACT), an educational network within the World Organization of Family Doctors (WONCA) Europe, outlined key principles for CME for general practitioners in its evidence-based report titled "Current State of the Evidence-Base for Effective Continuous Medical Education for Family Medicine and General Practice." Among other recommendations, the report

emphasizes that CME should be grounded in relevant learning needs and delivered using methods that actively engage practitioners and promote reflection.⁹

Geographical isolation remains a significant challenge in the provision of CPD for rural GPs. E-learning, also known as distance learning, offers a viable solution to this issue and is increasingly recognized as a preferred modality among rural healthcare professionals.⁸

E-learning encompasses a diverse range of formats, including synchronous and asynchronous delivery, interactive modules, modules of varying durations and formats, and both accredited and non-accredited programs. This diversity complicates efforts to categorize or evaluate e-learning uniformly. Therefore, a good starting point would be to understand the specific preferences of rural GPs regarding the content, format, and timing of e-learning, as this would indicate whether it is relevant, accessible, and potentially more impactful. It has been demonstrated that activities with participant and peer engagement tend to have a higher impact on professional practice.⁹ A survey among GPs practicing in Ireland showed that CPD activities that encourage peer engagement and interaction are considered of most benefit to patient care and that GPs appear to be adept at selecting CPD activities that suit their practice needs and translating this into enhanced patient care.¹⁰

Encouragingly, evidence suggests that e-learning, which offers greater flexibility and accessibility, is at least as effective as traditional face-to-face learning methods. Moreover, it has demonstrated superiority over no instruction in enhancing healthcare professionals' knowledge and behavior, thereby supporting its integration into rural medical education strategies.^{11,12} Technology-based delivery options are useful; however, the complex interrelatedness of time, use, travel, location, costs, interactivity, learning outcomes, and educational design suggests a need for careful consideration by educational providers.¹³

Objectives

The objectives of the survey were to assess rural GPs' current engagement with and awareness of e-learning resources relevant to their rural primary care context; identify rural GPs' preferences regarding the format, duration, and delivery of e-learning; explore rural GPs' perceived

learning needs and priority topics for rural primary care; and generate preliminary insights to inform the future development of context-appropriate e-learning provision for rural GPs.

Materials and methods

This was a descriptive learning-needs assessment based on a single survey questionnaire distributed to rural GPs through 2 organizations: The European Rural and Isolated Practitioners Association (EURIPA), which is a network of the European branch of WONCA, and the Royal College of General Practitioners (RCGP) in the UK, to understand their learning needs as rural GPs and to inform future educational planning (Table 1).

In this study, no formal geographic classification or population-density threshold was used to determine rurality. Respondents self-identified as working in rural primary care settings. The survey collected information from these self-defined rural GPs on awareness and use of rural primary care-relevant e-learning, preferred formats and durations, and perceived priority topics for CPD. Rural primary care-relevant e-learning was defined as online CPD designed for or directly relevant to the rural primary care context. This included e-learning addressing clinical, organizational, and professional challenges commonly encountered by rural GPs. It did not refer simply to any online CPD accessible to rural clinicians.

The survey did not collect any demographic data as the focus was on a general educational needs assessment. The survey was conducted in English as this is the formal language of both organizations. It included closed-response items and optional free-text fields. The survey was developed by expert consensus approach among scientific and executive board members of both organizations and was not piloted or validated before distribution. For the RCGP sample, the survey was distributed after an event organized by the RCGP Rural Forum and was explicitly addressed to rural GPs. For the EURIPA sample, respondents were members of a rural-practice network. This project was undertaken as a survey to understand members' learning needs within RCGP and EURIPA. In line with UK Health Research Authority (HRA) guidance, learning needs assessments or service evaluations

do not require review by a Research Ethics Committee. Survey participation was voluntary, no identifiable data were collected, and all responses were anonymized and processed in accordance with GDPR. Completion of the survey implied consent.

The RCGP sent out the survey as part of an evaluation questionnaire following a webinar on emergency care in October 2024. The survey was separate from the evaluation survey for the emergency webinar. Participation was voluntary. The RCGP used its internal survey platform. EURIPA members responded to an online, voluntary survey using Google Forms, distributed via the EURIPA newsletter and Google Group, with a reminder sent 4 weeks after the survey opened. The survey was open from 11 October to 22 December 2024. Quantitative data were summarized as percentages, and optional free-text responses were reviewed descriptively. No identifiable data were collected.

Results

A total of 187 rural GPs participated voluntarily: the RCGP sample comprised 157 respondents (as the total number of webinar attendees was not recorded, a response rate cannot be calculated), and 30 responses were received from 94 active EURIPA members (30/94, 32%).

Together, these samples represent convenience samples of rural GPs affiliated with either the RCGP or EURIPA rather than population-based cohorts. Responses from both recruitment streams were combined for analysis because all participants completed the same survey questionnaire.

When asked about awareness and use of rural primary care-relevant e-learning, defined as online CPD designed for or directly relevant to rural primary care rather than general online CPD, the majority (120, 64%) did not answer the question. Of the 67 out of 187 respondents who answered, 5 (8%) reported using rural primary care-specific e-learning (Table 2). The high non-response rate limits conclusions regarding overall awareness and use.

Fifty percent (93/187) of respondents favored 20–40 min, with 30–40 min being the clear preference (60 responses, 32%) for e-learning session length. Preferences for e-learning formats yielded 485 selections from 187 respondents

Table 1. Survey samples and recruitment

Group	Population/setting	Recruitment method	Number of respondents	Participation rate
RCGP (UK)	Royal College of General Practitioners (RCGP) members self-defining as rural GPs attending a webinar organized by the RCGP Rural Forum	link embedded in the post-webinar evaluation questionnaire	157	not reported
EURIPA (Europe)	Members of the European Rural and Isolated Practitioners Association (EURIPA), n = 94	online survey via newsletter and Google Group	30	32% (Note: no data available on how many members opened the Google group email or read the newsletter)
Total	Rural GPs affiliated with RCGP/EURIPA	as above	187	not applicable

Table 2. Awareness and use of rural primary care-relevant e-learning

Response category	n	% of respondents
Yes, I use rural-specific primary care e-learning	5	3
No, I do not use rural-specific primary care e-learning	62	33
Did not answer	120	64
Total	187	100

Percentages are rounded to whole numbers.

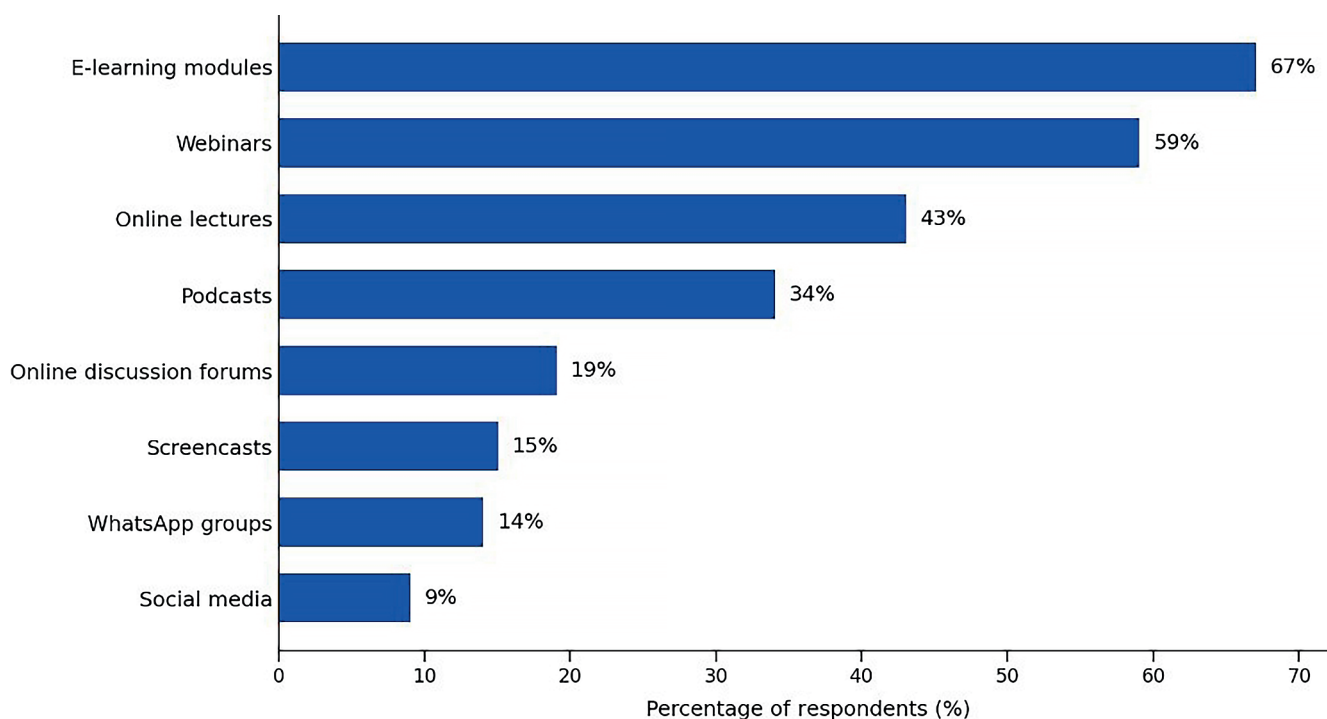


Fig. 1. Preferred e-learning formats. Multiple responses were allowed; percentages are calculated using the total number of respondents and therefore do not sum to 100

(Fig. 1). The most frequently selected formats were e-learning modules (125, 67%), webinars (110, 59%), and online lectures (81, 43%). Podcasts (63, 34%) and online discussion forums (36, 19%) were selected less often, while screencasts (28, 15%), WhatsApp groups (26, 14%), and social media (16, 9%) were the lowest-ranked options.

Respondents identified a wide range of clinical and non-clinical topics that they considered relevant to rural primary care e-learning, resulting in 1,011 topic selections (Fig. 2). The highest-ranked topics were emergency care (117 selections, 63%), medical complexity (97, 52%), and managing risk and uncertainty in rural practice (94, 50%). Addiction and mental health (90, 48%), frailty (90, 48%), telemedicine and remote consulting or assessment (71, 38%), and long-term conditions (69, 37%) were also prominent. Topics of lower priority included deprivation and rural poverty (62, 33%), loneliness and social medicine (58, 31%), conflict resolution and de-escalation (50, 27%), caring for temporary residents or tourists (48, 26%), and prevention of burnout (48, 26%). The lowest-ranked topics were agri-health (41, 22%), community orientation and social prescribing (41, 22%), and multidisciplinary working (35, 19%).

Open-text responses provided further insight into respondents' preferences. Suggestions included content addressing the social context of rural general practice, professional–personal boundary challenges, and the importance of platforms accessible across Europe. Respondents emphasized the value of interactive, case-based formats; opportunities for peer support (particularly for early-career or isolated primary care practitioners); skills-focused modules such as near-patient diagnostics (e.g., point-of-care ultrasound); hybrid learning options; and signposting to existing high-quality resources. Additional topics included supervision and teaching in rural practice, climate-related health impacts, and short observational visits to rural colleagues in low- or middle-income countries.

Discussion

The survey findings indicate that most respondents are interested in using interactive e-modules, webinars, and online lectures for their CPD. The preferred session length was 30–40 min, with a focus on topics relevant to rural

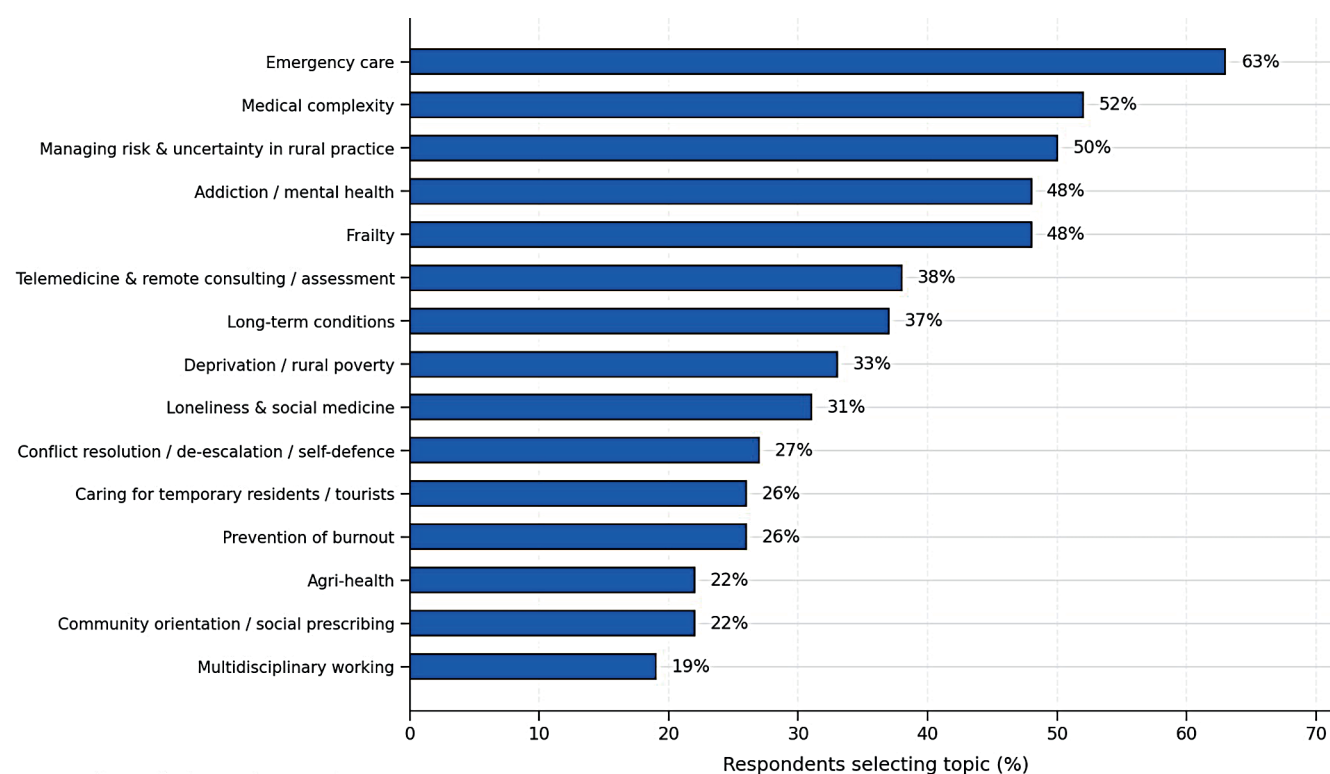


Fig. 2. Priority topics for rural primary care e-learning. Multiple responses were allowed; percentages are calculated using the total number of respondents and therefore do not sum to 100%

general practice, such as emergency care, multimorbidity and complexity, managing risk and uncertainty, mental health and addiction, frailty, and telemedicine. This aligns with EURACT's guidance, which emphasizes that CPD should be interactive, address relevant topics, and incorporate reflective practice to positively influence clinical behavior.⁹ Incorporating interactive elements into e-learning not only supports behavioral change but also helps mitigate burnout among practitioners.⁸

Although this descriptive, UK-weighted sample cannot establish Europe-wide priorities, the findings align with literature describing shared challenges in rural primary care. These include service delivery in resource-constrained environments, limited access to specialist care, geographical barriers to care for patients and practitioners, and the need for a broad, high-level clinical skill set to manage risk, uncertainty, and increasingly complex multimorbid aging populations.⁵ This supports the development of coordinated CPD resources for GPs at a pan-European or even global level through EURIPA or Rural WONCA, for example. These could include CPD resources on how to adapt as a clinician to resource constraints, develop a high tolerance for uncertainty and risk, expand the scope of practice to address multimorbidity in an elderly population and agriculture-specific occupational issues, respond to emergencies, and be skilled in telemedicine to overcome geographical barriers.

However, while certain rural challenges are common across countries, the implementation of more practice-based

or local and national solutions is influenced by local systems, regulations, language, and care infrastructure that are particularly relevant to rural primary care in these different national contexts; therefore, some CPD content is more country-specific and best delivered through national CPD platforms. The study cannot support country-level conclusions or recommendations because nationality- or country-level data were not collected or reported.

Efforts are underway to secure a platform and appropriate funding to develop e-learning modules focused on rural primary care in the UK. This is also currently happening at a more global level, initiated by Rural WONCA.

Limitations of the study

The study used 2 recruitment streams for the distribution of the same survey questionnaire to ensure an acceptable response rate and draw general observations on the educational needs of rural GPs that are contextually relevant to rural settings. Survey participants from the RCGP, and therefore from the UK, were disproportionately represented, accounting for 157/187 respondents, resulting in the combined results being heavily UK-weighted. The RCGP survey was sent out alongside the evaluation of a webinar on emergency care, which could have influenced responses regarding topic selection. Both samples were convenience samples and subject to selection bias, as participants were volunteers and likely to be individuals already highly engaged and proactive in their CPD. Therefore, the results should be

interpreted as descriptive. A further limitation of the study is that it does not investigate educational effectiveness and implementation feasibility.

Conclusions

Use of rural primary care-specific e-learning was reported by only a small number of respondents; however, high item nonresponse limits conclusions regarding overall awareness, availability, and use. E-learning could potentially bridge a gap in provision. Rural GPs expressed a preference for e-learning in a structured format, such as e-modules, with an interactive element, such as webinars. Rural GPs particularly value content that covers both clinical and non-clinical topics relevant to rural primary care, such as emergency care and the management of complexity, risk, and uncertainty. The study highlights the need for further research into which e-learning resources relevant to rural GPs could be developed and delivered on a European or global scale, and which would be better suited to local or national learning platforms.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.21373992>. The package contains the following files.

Supplementary Table 1. Preferred duration of e-learning sessions.

Supplementary Table 2. Preferred e-learning formats.

Supplementary Table 3. Survey questions.

Supplementary Table 4. Priority topics for rural e-learning.

Data Availability Statement

Data sharing does not apply to this article, as all data are already included in the manuscript and its supplementary files.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Genetic evidence linking gout to increased risk of epilepsy: A two-sample Mendelian randomization study

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Abstract

Background. While observational studies have explored the association between gout, uric acid (UA) levels, and the risk of epilepsy, their findings remain inconsistent.

Objectives. To clarify the potential causal relationship, we performed a Mendelian randomization (MR) analysis.

Materials and methods. We conducted a bidirectional MR analysis using genome-wide association study (GWAS) summary data. Epilepsy data were sourced from the International League Against Epilepsy (ILAE), gout data from the FinnGen consortium and a GWAS of clinically defined gout and its subtypes, and UA data from the BioBank Japan (BBJ) and a genome-wide meta-analysis of serum UA levels in Japanese individuals. The inverse-variance weighted (IVW) method served as the primary causal inference approach. To ensure robustness, we complemented this with 3 additional methods: MR-Egger, weighted median, and MR using Robust Adjusted Profile Score (MR-RAPS) – the latter specifically employed to account for potential weak instrument bias due to the limited number of single-nucleotide polymorphisms (SNPs) included in this analysis.

Results. Our analysis identified a causal association between genetically predicted gout and an increased risk of epilepsy (odds ratio (OR) = 1.014, 95% confidence interval (95% CI): 1.011–1.017, $p < 0.001$), generalized epilepsy (OR = 1.009, 95% CI: 1.000–1.018, $p = 0.042$), and focal epilepsy (OR = 1.044, 95% CI: 1.002–1.088, $p = 0.041$) in Europeans. In contrast, UA levels showed no significant association with epilepsy or its subtypes in this population. Similarly, neither gout nor UA levels were linked to epilepsy in East Asians. Sensitivity analyses generally supported the robustness of the primary findings.

Conclusions. This MR study provides genetic evidence supporting a potential causal role of gout in increasing the risk of epilepsy, including generalized and focal epilepsy, in European populations. In contrast, genetically predicted UA levels showed no significant association with epilepsy. These findings support a potential role of gout in epilepsy risk and warrant further investigation into the underlying mechanisms.

Key words: gout, epilepsy, Mendelian randomization analysis, uric acid metabolism, genetic predisposition to disease

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Highlights

- Mendelian randomization (MR) analysis reveals a causal link between gout and increased epilepsy risk, including both generalized and focal epilepsy, in European populations.
- Genetically predicted serum uric acid (UA) levels show no causal association with epilepsy, indicating that gout-related mechanisms act independently of UA.
- Bidirectional MR using large genome-wide association study (GWAS) datasets confirms robust findings without pleiotropy or reverse causation, strengthening genetic evidence for gout as a neurological risk factor.
- These population-specific genetic insights position gout as a potential biomarker and therapeutic target for epilepsy risk stratification, emphasizing the need for precision medicine approaches.

Background

Epilepsy is a brain disorder defined by meeting any of the following criteria: 1) at least 2 unprovoked seizures occurring more than 24 h apart; 2) 1 unprovoked seizure with a high subsequent recurrence risk ($\geq 60\%$); or 3) a diagnosis of an epilepsy syndrome in individuals with a history of unprovoked seizures over a decade.¹ Epilepsy is estimated to affect more than 70 million people globally, imposing a substantial global economic and social burden.² Epilepsy can arise from various etiologies, including structural, infectious, genetic, immune-mediated, and metabolic factors, among others. Identifying the underlying cause of epilepsy and targeting it with precise treatment is crucial. A metabolic etiology denotes a well-defined metabolic deficiency that manifests as systemic biochemical alterations, encompassing conditions such as porphyria, uremia, aminoacidopathies, or pyridoxine-dependent seizures.³ However, there remain numerous unknowns in this field.

Gout is the most prevalent form of inflammatory arthritis, resulting from abnormal purine metabolism and impaired uric acid (UA) excretion. It manifests as elevated serum UA levels and joint pain. When the UA concentration in the blood becomes excessively high, monosodium urate (MSU) crystals can form, potentially leading to severe pain and joint inflammation. Monosodium urate crystals activate the immune response in the body, activating monocyte or macrophage inflammatory mediators and leading to an inflammatory response in the joint. Two population-based cohort studies conducted in German and Chinese populations found that gout patients have a significantly increased risk of epilepsy compared to those who do not suffer from gout.^{4,5} Animal experiments have also shown that hyperuricemia may facilitate seizure generalization, whereas a reduction in UA levels can produce anticonvulsant effects.⁶ However, the mechanism by which gout or hyperuricemia induces epilepsy is still elusive. Given the susceptibility of traditional epidemiological studies to confounding and reverse causality, the relationship between gout, UA levels, and epilepsy risk remains unclear. To address this, we employed Mendelian randomization

(MR) to assess their causal associations at the genetic level using single-nucleotide polymorphisms (SNPs).

Mendelian randomization employs genetic variants as instrumental variables to infer causal relationships between exposures and outcomes. A key advantage of this approach is that, since genetic alleles are randomly assigned and fixed at conception, MR estimates are less susceptible to confounding biases and reverse causation than conventional observational studies.^{7,8} Moreover, because the genetic code is not influenced by environmental factors or pre-clinical diseases, it is also less susceptible to bias caused by reverse causation. Recently, MR has also been used to explore the potential etiology of epilepsy.^{9,10} Large-scale genome-wide association studies (GWAS) have identified multiple susceptibility loci and highlighted diverse biological mechanisms underlying the common epilepsies.¹¹

Objectives

Therefore, this two-sample MR study aims to investigate the causal effects of gout and UA levels on the risk of epilepsy and its subtypes. We want to provide an evidence base for epilepsy prevention and the screening of high-risk groups.

Materials and methods

Study overview

We conducted a two-sample MR analysis using publicly available GWAS summary statistics to assess the causal effects of gout and UA on various epilepsy phenotypes (including generalized and focal subtypes) in European populations (the study design is outlined in Fig. 1). The MR methodology is grounded on 3 key assumptions: 1) the genetic instrumental variables (IVs) must be strongly associated with the exposure; 2) the IVs are independent of confounders; and 3) the IVs influence the outcome solely via the exposure.

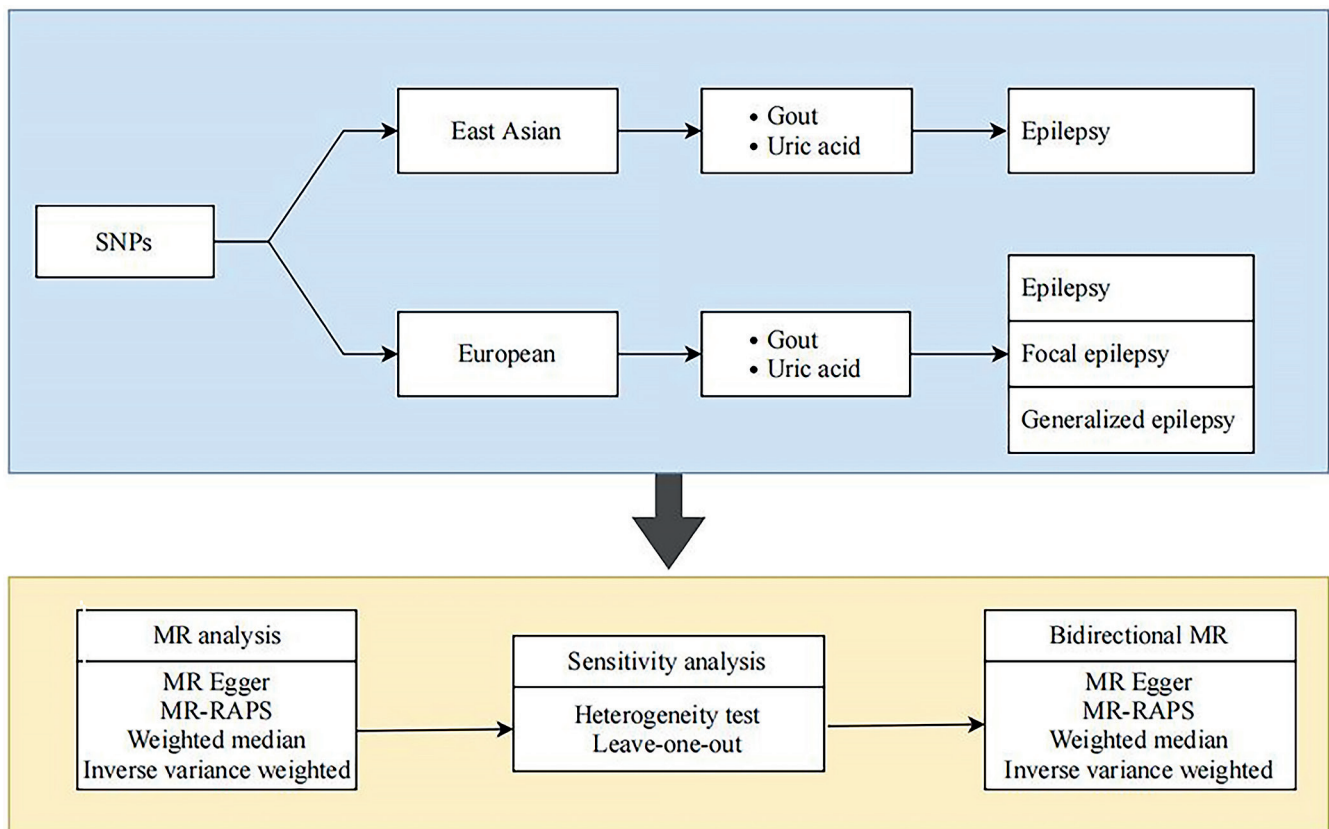


Fig. 1. Study design and core assumptions of Mendelian randomization (MR): data sources and study design of our bidirectional MR analysis

SNPs – single-nucleotide polymorphisms.

Table 1. Detailed characteristics of genome-wide association study (GWAS) datasets used to select instrumental variables for Mendelian randomization (MR) analysis

Study name	Trait	Consortium	Population	Sample size	SNPs ($p < 5 \times 10^{-8}$)	LD
finn-b-M13_GOUT	gout	FinnGen	European	N/A	1451	9
Nakayama et al., 2017 ¹²	gout	N/A	East Asian	N/A	65	49
	unit decrease	N/A		N/A	30	19
	unit increase	N/A		N/A	35	30
	uric acid	N/A		N/A	32	20
bbj-a-57	uric acid	BioBank Japan (BBJ)	East Asian	109,029	32	20
Nakatochi et al., 2019 ¹³	uric acid	N/A	East Asian	121,745	20	N/A

SNP – single-nucleotide polymorphism; LD – number of SNPs removed due to linkage disequilibrium during clumping ($r^2 > 0.01$ within 1000 kb); N/A – not applicable.

Data sources for the exposure

Exposure data for genetic variants associated with gout in European populations were obtained from FinnGen, which identified 1,451 gout SNPs ($p < 5 \times 10^{-8}$). After clumping ($r^2 < 0.01$, kb = 1,000), 3 independent SNPs were retained as IVs for the MR analysis (Table 1).^{12,13} Meanwhile, IVs for gout in East Asian populations were obtained from the study by Nakayama et al.,¹² which identified 65 gout-related SNPs ($p < 5 \times 10^{-8}$). After clumping, 30 independent SNPs with a unit decrease and 35 with a unit increase were adopted as IVs for gout (Table 1).

For UA, an analysis of BioBank Japan (BBJ) data from East Asian populations (Table 1)^{12,13} identified 32 independent

SNPs as IVs for UA. Additionally, a separate East Asian GWAS (Nakatochi et al.¹³) was included in the UA analyses, with 10–20 SNPs included in the final MR analyses depending on the epilepsy outcome (Table 1).¹³

Data sources for the outcome

Data for this study were derived from publicly available GWAS summary statistics provided by the International League Against Epilepsy (ILAE) consortium. These studies primarily included individuals of European ancestry. The analysis incorporated 3 phenotypes: all epilepsy (15,212 cases and 29,677 controls), generalized epilepsy (3,769 cases and 29,677 controls), and focal epilepsy (9,671

Table 2. Characteristics of the included genome-wide association study (GWAS) summary-level data on epilepsy, gout, and uric acid (UA)

ID	Trait	Consortium	Study name	Population	Sample size	Case	Controls
ieu-b-8	epilepsy, all documented cases	International League Against Epilepsy (ILAE)	Nakayama et al., 2017 ¹²	European	44,889	15,212	29,677
ieu-b-9	generalized epilepsy, all documented cases		Nakayama et al., 2017 ¹²	European	33,446	3,769	29,677
ieu-b-10	focal epilepsy, all documented cases		Nakayama et al., 2017 ¹²	European	39,348	9,671	29,677
bbj-a-115	epilepsy	BioBank Japan (BBJ)	N/A	East Asian	212,453	2,143	210,310

cases and 29,677 controls) (Table 2). For East Asian populations, epilepsy data were obtained from BBJ (2,143 cases and 210,310 controls) (Table 2). As this research utilized only de-identified, aggregate data from published studies, no additional ethical approval was required. Ethical approvals for the original GWAS were obtained, as reported in their respective primary publications.

Instrumental variable selection

To select IVs, SNPs significantly associated with gout and UA (or with epilepsy in reverse-direction analyses) were initially identified at a genome-wide significance threshold ($p < 5 \times 10^{-8}$). Linkage disequilibrium (LD) among these SNPs was mitigated by applying a clumping procedure ($r^2 < 0.01$, distance = 1,000 kb). Each candidate SNP was further evaluated for potential violations of MR assumptions (particularly independence from confounders and absence of direct pathways to the outcome) using the PhenoScanner database (<http://www.phenoscanter.medschl.cam.ac.uk>).¹⁴ Palindromic SNPs and those absent from the outcome dataset were excluded. The strength of the selected instruments was quantified using the F-statistic; an F-statistic exceeding 10 was taken to indicate minimal risk of weak instrument bias.¹⁵

Functional enrichment analysis

Functional enrichment analysis, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses, was performed using the DAVID Functional Annotation Tool (<https://david-bioinformatics.nih.gov>). The results were visualized using the SRplot online bioinformatics platform (<https://www.bioinformatics.com.cn>). Gene Ontology terms were classified into 3 domains: biological process (BP), cellular component (CC), and molecular function (MF). Enrichment was considered significant at $p < 0.05$.

Statistical analyses

To analyze the causal associations, we employed multiple MR methods. The inverse-variance weighted (IVW) method under a random-effects model served as the primary analysis. Sensitivity analyses were conducted using

the weighted median method and MR-Egger regression, the latter of which also assessed potential pleiotropy. Given the limited number of genetic instruments, Mendelian Randomization using Robust Adjusted Profile Score (MR-RAPS) was additionally applied to account for potential weak instrument bias. All causal estimates are presented as odds ratios (ORs) with 95% confidence intervals (95% CIs). These analyses were implemented using the TwoSampleMR package v. 4.4.1 (<https://mrcieu.github.io/TwoSampleMR>) in R (R Foundation for Statistical Computing, Vienna, Austria).

Results

Selection of genetic variants

The validity of the MR assumptions was rigorously tested. The IVs exhibited strong correlation with the exposures (F-statistics > 10; Table 3), satisfying the 1st key assumption. The MR-Egger intercept test detected no significant pleiotropy for most outcomes (all $p > 0.05$), except for the analysis of UA (unit increase) and generalized epilepsy ($p = 0.042$). In contrast, the corresponding MR-Egger intercept test for gout and generalized epilepsy was not significant ($p = 0.932$). These results largely satisfied the 2nd assumption. Furthermore, no heterogeneity was detected using Cochran's Q test (all $p > 0.05$; Table 3), thus fulfilling the 3rd assumption.

Mendelian randomization analysis

After data preprocessing, we performed MR analyses to assess causal relationships between the exposures (gout, UA) and epilepsy using IVW, weighted median, and MR-Egger methods. At the adjusted significance threshold, the IVW method revealed 3 significant associations between gout and epilepsy (Fig. 2). Specifically, gout showed a positive association with epilepsy risk in European populations (OR = 1.014, 95% CI: 1.011–1.017, $p < 0.001$). A similar trend was observed with the weighted median method, though with a wider CI (OR = 1.014, 95% CI: 0.988–1.040, $p = 0.306$). Moreover, MR-Egger intercept tests indicated no significant directional pleiotropy ($p > 0.05$), supporting the robustness of the primary findings.

Table 3. Results of hypothesis testing

Trait	Strength	MR-Egger intercept test		Heterogeneity test			
	F value	Intercept	p-value	Q Egger	p-value	Q IVW	p-value
European							
Gout							
Epilepsy	121.08	−0.0025	0.903	0.0041	0.949	0.0275	0.986
Generalized epilepsy	121.08	−0.0021	0.932	0.1795	0.672	0.1911	0.909
Focal epilepsy	121.08	0.0096	0.841	1.6977	0.193	1.8084	0.405
Uric acid							
Epilepsy							
Unit decrease	399.19	0.0038	0.327	2.5854	0.958	3.6761	0.931
Unit increase	99.81	−0.004	0.367	7.1857	0.708	8.0778	0.706
Generalized epilepsy							
Unit decrease	318.86	0.0042	0.269	6.0315	0.871	7.3844	0.831
Unit increase	85.25	−0.0097	0.042	11.1548	0.742	16.0789	0.448
Focal epilepsy							
Unit decrease	340.11	−0.0031	0.592	7.2868	0.698	7.594	0.749
Unit increase	82.90	−0.0009	0.892	12.1553	0.791	12.1744	0.838
East Asian							
Gout							
Epilepsy	51.42	−0.0088	0.844	4.7082	0.582	4.7506	0.690
Uric acid							
Epilepsy	180.33	−0.0002	0.981	29.859	0.473	29.8596	0.525

MR – Mendelian randomization; IVW – inverse-variance weighted.

Genetically predicted gout was found to be associated with an elevated risk of generalized epilepsy in European populations. Specifically, the OR for generalized epilepsy was 1.009 (95% CI: 1.000–1.018, $p = 0.042$), as illustrated in Fig. 2. Consistent trends were observed using the weighted median method, albeit with broader CIs (OR = 1.011; 95% CI: 0.981–1.041, $p = 0.486$). Notably, for gout and generalized epilepsy, the MR-Egger intercept test provided no indication of directional pleiotropy ($p = 0.932$), suggesting that the observed association was not confounded by horizontal pleiotropy.

Additionally, genetically predicted gout was associated with an increased risk of focal epilepsy in European populations (OR = 1.044, 95% CI: 1.002–1.088, $p = 0.041$; Fig. 2). A consistent, though nonsignificant, trend was observed with the weighted median method (OR = 1.038, 95% CI: 0.992–1.086, $p = 0.111$). The absence of directional pleiotropy in the MR-Egger intercept test for gout and focal epilepsy ($p = 0.841$) further supported this association. In contrast, neither UA levels nor gout showed any significant causal relationship with epilepsy or its subtypes in East Asian populations (Fig. 3).

Reverse Mendelian randomization analysis

We also performed reverse MR analysis to assess the causal effect of epilepsy and its subtypes on gout in European populations. Genetically predicted epilepsy,

generalized epilepsy, and focal epilepsy all showed no significant association with gout risk (IVW ORs: 1.082, 1.083, and 0.991, respectively; all $p > 0.05$; Fig. 4). These null findings were consistent with those obtained using the weighted median method, and the absence of directional pleiotropy (MR-Egger intercept tests: $p > 0.05$) supported the robustness of these results.

Sensitivity analysis

In the leave-one-out sensitivity analysis, after each SNP was removed in turn, the beta estimate based on the remaining SNPs consistently remained to the right of the null line ($\beta = 0$), indicating a positive causal effect (Fig. 5). This further demonstrates the robustness of the results.

Gene set enrichment analyses

To explore the potential mechanisms linking gout to epilepsy, we performed functional enrichment analyses on 2 candidate genes using the DAVID Functional Annotation Tool. The GO analysis categorized gene functions into 3 domains: molecular function, cellular component, and biological process. Concurrently, pathway enrichment was assessed using the KEGG database. The GO results indicated that the genes were primarily associated with the apical plasma membrane, brush border membrane, and the apical part of the cell at the cellular component

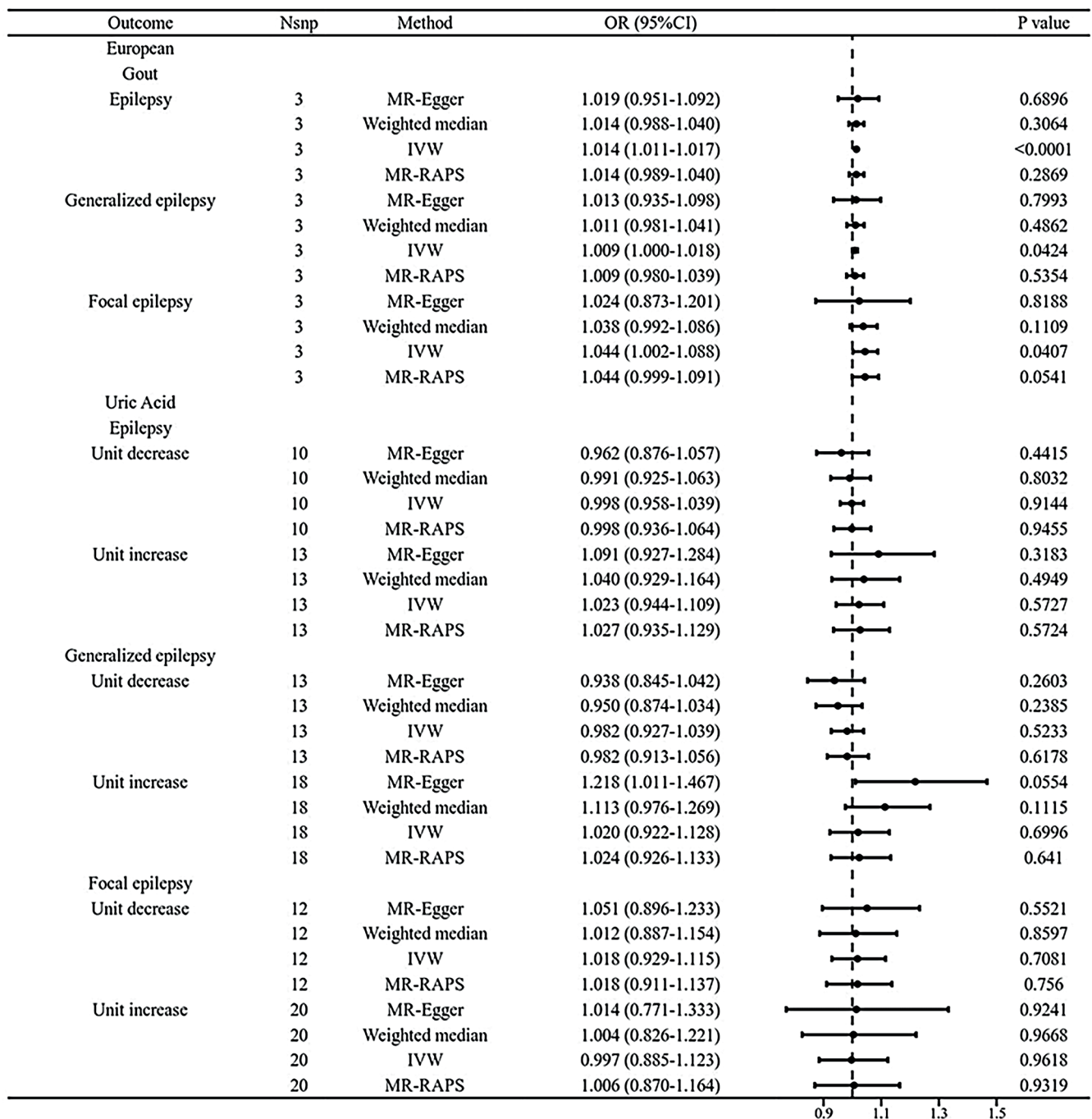


Fig. 2. Association of genetically predicted gout and uric acid (UA) levels with epilepsy in Europe using 4 different Mendelian randomization (MR) methods: inverse-variance weighted (IVW), MR-Egger, weighted median, and Mendelian Randomization using Robust Adjusted Profile Score (MR-RAPS). Positive correlations between gout and epilepsy, between gout and generalized epilepsy, and between gout and focal epilepsy were suggested. There is no correlation between UA and epilepsy, between UA and generalized epilepsy, or between UA and focal epilepsy

OR – odds ratio; 95% CI – 95% confidence interval.

level. For molecular function, they were enriched in ABC-type xenobiotic transporter activity and efflux transmembrane transporter activity. Biologically, the genes were implicated in the urate metabolic process, organic anion transport, and renal tubular secretion (Fig. 6). The KEGG pathway analysis further revealed significant enrichment in antifolate resistance, ABC transporters, and bile secretion (Fig. 6).

Discussion

Despite limited research on the link between gout and epilepsy, our investigation sought to elucidate the causal association between gout and epilepsy risk using an MR design. Our findings indicated that genetically predicted gout was associated with an elevated risk of epilepsy in European populations. Specifically, individuals with a genetically

Outcome	Nsnp	Method	OR (95%CI)	P value
East Asian				
Gout				
Epilepsy	8	MR-Egger	0.986 (0.792-1.229)	0.9062
	8	Weighted median	0.988 (0.904-1.080)	0.7946
	8	IVW	0.965 (0.913-1.020)	0.2076
	8	MR-RAPS	0.964 (0.900-1.034)	0.3057
Uric Acid				
Epilepsy	32	MR-Egger	0.918 (0.691-1.220)	0.5607
	32	Weighted median	0.894 (0.678-1.178)	0.4263
	32	IVW	0.916 (0.759-1.105)	0.358
	32	MR-RAPS	0.915 (0.755-1.110)	0.3674

Fig. 3. Association of genetically predicted gout and uric acid (UA) levels with epilepsy in East Asia using 4 different Mendelian randomization (MR) methods: inverse-variance weighted (IVW), MR-Egger, weighted median, and Mendelian Randomization using Robust Adjusted Profile Score (MR-RAPS). Neither gout nor UA is correlated with epilepsy, generalized epilepsy, or focal epilepsy

OR – odds ratio; 95% CI – 95% confidence interval.

Exposure	Nsnp	Method	OR (95%CI)	P value
Epilepsy	43	MR-Egger	1.093 (0.448-2.670)	0.846
	43	Weighted median	1.038 (0.872-1.235)	0.6742
	43	IVW	1.083 (0.979-1.199)	0.1217
	43	MR-RAPS	1.086 (0.956-1.232)	0.205
Generalized epilepsy	34	MR-Egger	0.971 (0.548-1.720)	0.9207
	34	Weighted median	1.063 (0.786-1.437)	0.6924
	34	IVW	0.991 (0.827-1.187)	0.919
	34	MR-RAPS	0.990 (0.796-1.233)	0.9312
Focal epilepsy	52	MR-Egger	0.971 (0.548-1.720)	0.5375
	52	Weighted median	1.063 (0.786-1.437)	0.9951
	52	IVW	0.991 (0.827-1.187)	0.3135
	52	MR-RAPS	0.990 (0.796-1.233)	0.4437

Fig. 4. Association of genetically predicted gout with epilepsy in Europe using 4 different Mendelian randomization (MR) methods: inverse-variance weighted (IVW), MR-Egger, weighted median, and Mendelian Randomization using Robust Adjusted Profile Score (MR-RAPS). No correlations were suggested between gout and epilepsy, between gout and generalized epilepsy, or between gout and focal epilepsy

OR – odds ratio; 95% CI – 95% confidence interval.

predicted propensity for gout exhibited a higher likelihood of developing epilepsy. To the best of our knowledge, this is the first MR study to provide genetic evidence supporting a causal association between gout and epilepsy risk.

The findings presented herein align with the outcomes derived from 2 cohort studies sourced from the Registry of Catastrophic Illnesses Patient Database (RCIPD)⁵ and the IQVIA Disease Analyzer data source.⁴ The former cohort encompassed 104,238 individuals aged 20 years or older, and non-gout controls were frequency-matched to the gout group in a ratio of 2 : 1, considering factors such as demographic and clinical characteristics. The analysis revealed that the gout group exhibited a 1.27-fold increase in the overall crude hazard ratio (HR) for epilepsy, in contrast to the control group. After the analyses were adjusted for potential confounding variables, including age, sex, and comorbidities, the gout patients continued to demonstrate a significantly increased risk of epilepsy compared to the control group. Specifically, the adjusted

HR for epilepsy among gout patients was 1.25 (95% CI: 1.15–1.36). Similarly, our findings are consistent with those of a retrospective cohort analysis that used the IQVIA Disease Analyzer database and involved 224,964 primary care outpatients.⁴ That study identified 112,482 outpatient-managed gout patients and matched them 1 : 1 with non-gout individuals based on sex, age, annual consultation frequency, and pre-existing epilepsy risk factors. Over a 10-year follow-up, epilepsy incidence was significantly higher in the gout cohort (2.2%) than in the non-gout cohort (1.6%; log-rank $p < 0.001$). These retrospective studies consistently indicate that gout can elevate the risk of epilepsy, aligning with our research findings. However, these retrospective studies are confined to basic demographic information and lack detailed information on the etiology, seizure types, and treatment modalities of epilepsy. Furthermore, genetic factors have not been taken into consideration in these studies. Our research methodology is grounded in Mendel's laws of inheritance, utilizing

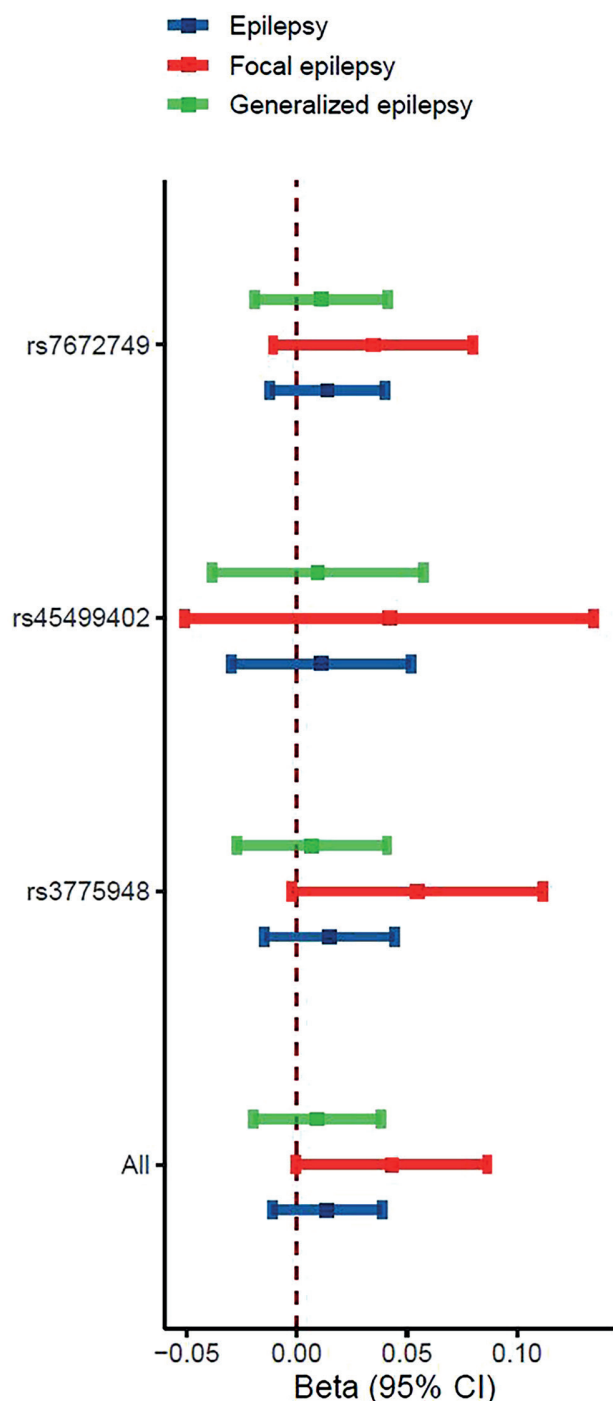


Fig. 5. Leave-one-out sensitivity analysis. Each black square represents the beta estimate obtained after sequentially removing one single-nucleotide polymorphism (SNP), with the horizontal line indicating the corresponding 95% confidence interval (95% CI). The vertical gray line represents the null value ($\beta = 0$)

genetic variations associated with gout as instrumental variables. By comparing epilepsy occurrence among individuals with various genotypes, we aimed to infer the impact of these genes on the occurrence of epilepsy. This approach enabled us to provide a more precise genetic foundation for future studies in transcriptomics, proteomics, or epigenomics, and furthermore, it holds promise for advancing targeted therapies for epilepsy.

What mechanisms might underlie the association between gout and an increased risk of epilepsy, and could genetic correlations contribute to these mechanisms? Obviously, gout, characterized as an immune-inflammatory disease, is recognized as a prototypical inflammatory arthritis intricately associated with disturbances in purine metabolism and impaired UA excretion, which promote the formation of MSU crystals.¹⁶ When blood UA concentrations surpass the solubility limit (typically exceeding 420 $\mu\text{mol/L}$), MSU crystals may precipitate.^{17,18} When MSU crystals deposit within joints and stimulate the synovial membrane,¹⁹ they can induce acute attacks of arthritis, manifesting as symptoms such as joint redness, swelling, warmth, and pain. Additionally, MSU crystals induce inflammatory reactions and oxidative stress²⁰ and increase neuronal excitability.²¹ The deposition of urate crystals triggers the NLRP3 inflammasome via caspase-1 activation, resulting in the release of pro-inflammatory cytokines such as interleukin (IL)-1 β and IL-18.^{22,23} These cytokines, particularly IL-1 β expressed by activated microglia and astrocytes, can modulate glutamatergic neurotransmission, thereby potentially playing a key role in the pathogenesis of epilepsy. Specifically, IL-1 β enhances the release of glutamate from astrocytes while concurrently reducing its reuptake, leading to increased glutamate availability within neuronal synapses. This increase in synaptic glutamate availability promotes neuronal hyperexcitability.²⁴ Furthermore, IL-1 β is hypothesized to induce seizures by upregulating postsynaptic NMDA receptors, specifically through activation of the GluN2B subunit, consequently enhancing glutamatergic signaling and potentially triggering seizure activity.²⁵ Therefore, gout-induced inflammation, oxidative stress, and increased neuronal excitability may contribute to seizure susceptibility.

Apart from oxidative stress, inflammation, and increased excitability of neurons, studies have indicated that pain itself can also induce epileptic seizures. Over the past decades, numerous studies have demonstrated the involvement of the cerebral cortex and its extensive cortical network in the processing of pain.²⁶ These investigations have revealed that the mechanism underlying seizures encompasses alterations in the circuitry interconnecting the thalamus and cerebral cortex.²⁶ Consequently, it is plausible that the pain associated with gout could provoke seizures by inducing aberrant electrical activity in these circuits. However, this specific link remains hypothetical, as no clinical or experimental studies have directly investigated whether gout-related pain can induce epilepsy, highlighting a valuable direction for future research.

The aforementioned discussion delves into the mechanisms by which gout can elicit inflammation, oxidative stress, and heightened neuronal excitability, either directly or indirectly, with hyperuricemia being a contributing factor in this process. Many studies indicate that UA can inhibit secondary injury and neuronal apoptosis through its antioxidant^{27,28} and neuroprotective effects,^{28,29} and excessive UA can lead to mitochondrial dysfunction, oxidative

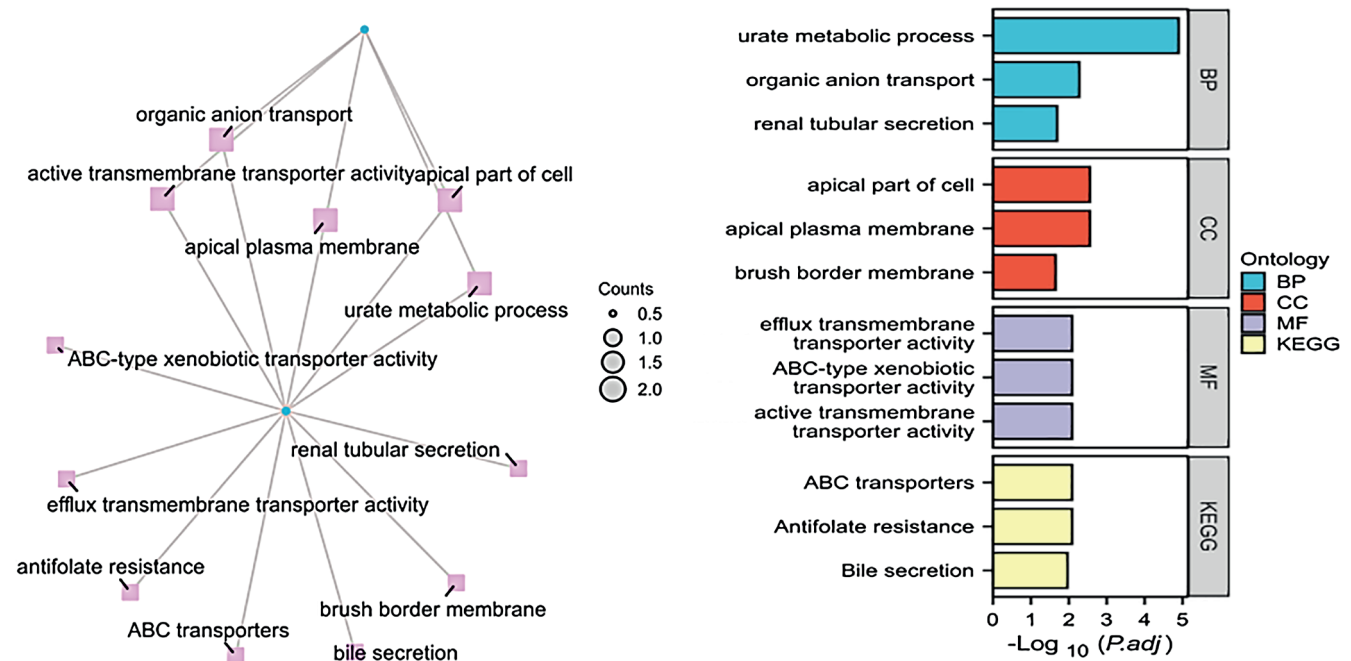


Fig. 6. Enrichment analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG)

stress,³⁰ and apoptosis in cells, which can increase oxidative damage to nerve cells.^{31,32} In our study, we observed no significant association between genetically predicted UA levels and the risk of epilepsy, generalized epilepsy, or focal epilepsy in European and East Asian populations. Lack of a linear association in our study may be explained by a U-shaped relationship, as research reports that both high (>322 $\mu\text{mol/L}$) and low (<248 $\mu\text{mol/L}$) serum uric acid (SUA) levels increase post-infarction epilepsy risk compared to moderate levels (248–322 $\mu\text{mol/L}$).⁸ This indirectly suggests that different UA levels may have different effects. Because UA levels were not provided in the European data, whether this relationship applies to our findings cannot be determined. The neuroprotective mechanism of UA in animal models has primarily been attributed to its antioxidant properties, which are often dependent on astroglia.^{33,34} This is exemplified by a study investigating the role of astrocytes in urate-mediated protection of dopaminergic cells in a model of Parkinson's disease (PD).³³ It was confirmed that UA had a neuroprotective effect in the co-culture of astrocytes and dopaminergic cells, reducing oxidative stress-induced cell death and protein damage. Meanwhile, UA has been demonstrated to reduce oxidative stress-induced neuronal damage. In the co-culture of spinal cord cells and astrocytes, UA can protect against glutamate-mediated neuronal injury.³⁴ Furthermore, hyperuricemic mice exhibited increased susceptibility to secondarily generalized tonic-clonic (SGTC) seizures; allopurinol treatment reduced the severity of these seizures.¹⁹ Given the potential mechanisms mentioned above and the fact that our study did not provide UA level data, we are unable to determine whether the lack of an association between UA and epilepsy in Asian populations can be explained by the antioxidant

effects of UA. Consequently, to elucidate the specific relationship between UA levels and epilepsy, there is an urgent need for more in-depth cohort studies and basic research to further validate and deepen our understanding.

Furthermore, ancestry-related factors may contribute to the observed differences, as genetically predicted gout was not associated with epilepsy in East Asian populations in our study. It is well-established that the German diet is characterized primarily by meat, cheese, bread, and beer, among other staples. Notably, meat and beer are considered high-purine foods. Could this dietary pattern explain the discrepancies observed in outcomes between Germans and East Asians? The dietary culture of East Asians is notably diverse, encompassing rice, noodles, vegetables, and meat as primary ingredients. Currently, there is a lack of research examining the relationship between gout and UA risk across different ancestry groups. However, upon reviewing the available literature, we found that greater adherence to the Southern European Atlantic Diet (SEAD) was associated with decreased concentrations of high-sensitivity C-reactive protein (hs-CRP), triglycerides, and insulin, as well as reduced insulin resistance, lower urine albumin levels, and decreased systolic blood pressure (SBP).³⁵ However, these observations do not adequately explain our research findings.

Beyond etiological and demographic considerations, our findings also prompt an evaluation of potential bidirectional interactions in clinical practice. Notably, anticonvulsant medications themselves are established modulators of SUA levels, creating a complex interplay with gout-related pathophysiology. An early clinical study demonstrated that long-term use of enzyme-inducing anti-seizure medications (ASMs) such as phenytoin, carbamazepine,

and primidone is associated with significantly lower SUA levels than those in both healthy controls and patients on valproate (who showed relatively higher levels within the treated cohort).³⁶ This differential effect is unlikely to be an artifact of drug monitoring, as assays for phenytoin and phenobarbital have been shown to be unaffected by high concentrations of UA.³⁷

Regarding the fundamental relationship between gout and UA, the question arises whether gout, characterized by elevated UA levels, possesses anti-epileptic properties or exacerbates epilepsy. Some cohort studies suggest that gout increases the risk of PD^{38,39} and dementia,⁴⁰ whereas others suggest a reduced risk^{41,42} or no association.⁴³ Meanwhile, gout also presents a paradoxical association with other neurological disorders, notably PD and dementia. Oxidative stress and inflammatory reactions represent one of the key pathogenic mechanisms underlying PD and dementia.^{44,45} This finding may offer a plausible explanation for the phenomenon that gout increases the risk of PD and dementia. Additionally, several cohort studies have confirmed that hyperuricemia may increase the risk of dementia and cognitive dysfunction.^{46–48} However, these studies have not demonstrated the presence of MSU crystal formation. Therefore, the observed associations can only be linked to hyperuricemia and cannot be definitively attributed to gout. Gout is characterized as an autoinflammatory disease. Therefore, gout may potentially be categorized as an immune-mediated condition. However, further basic research is required to investigate and validate this possibility.

The functional enrichment analysis identified 2 candidate genes, *ABCG2* and *SLC2A9*. *ABCG2*, an ATP-binding cassette transporter, has been implicated as a potential drug-related target in epilepsy.⁴⁹ Other studies have suggested that *ABCG2* polymorphisms are associated with resistance to lamotrigine and sodium valproate,^{50,51} as well as with urate levels and gout.^{52,53} *SLC2A9* encodes the UA/glucose transporter GLUT9, which is associated with hyperuricemia or gout.^{52–55} To date, no studies have directly linked *SLC2A9* to epilepsy.

Limitations of the study

Our study has several limitations. The study was based on GWAS data from individuals of European and East Asian ancestry, and therefore the results may not be generalizable to other populations. In addition, because the data were derived from GWAS summary statistics, we were unable to further explore causal associations in populations with different characteristics.

Conclusions

Our MR study reveals a distinct role for gout in elevating epilepsy risk among Europeans, while genetically predicted UA levels showed no significant association in either

European or East Asian populations. These findings suggest a potential epileptogenic role of gout, whereas no evidence of a causal association was observed for genetically predicted UA levels, warranting further investigation into the underlying mechanisms.

Collectively, current cohort studies investigating the associations among gout, UA levels, and epilepsy continue to be impacted by unpredictable confounding variables, which may account for the observed discrepancies in results. These discrepancies may arise from differences in study populations, including variations in race, age range, and gender of the analyzed cohorts, as well as inconsistencies in diagnostic criteria for epilepsy. Furthermore, methodological variations, such as the nature of the studies (prospective, retrospective, or case–control) and the potential factors adjusted for in the statistical models of prior research, may also contribute to these inconsistencies. Conducting retrospective analyses across multiple centers and diverse racial groups poses significant challenges. To address these issues, future research endeavors should delve deeper into the relationship between gout and epilepsy, as well as the underlying mechanisms, through cellular and animal experimentation. Such studies will provide a crucial foundation for advancing our understanding of the etiology, diagnosis, and treatment of epilepsy.

Collectively, these observations underscore that SUA monitoring and the differential metabolic profiles of ASMs could be relevant considerations in managing patients with epilepsy, particularly those with or at risk for hyperuricemia or gout. Future studies are needed to clarify how these drug-induced alterations in urate homeostasis might influence long-term neurologic or metabolic outcomes in epilepsy.

In contrast to traditional observational studies, this MR analysis utilized summary-level data from large-scale GWAS, thereby strengthening causal inference regarding the association between the exposures and epilepsy. The consistency of findings across multiple sensitivity analyses further supports the robustness of the results. A previous population-based cohort study also reported a positive association between gout and the risk of epilepsy (adjusted HR = 1.25; 95% CI: 1.15–1.36), after adjustment for age, sex, and comorbidities.⁵ As epilepsy remains a serious global neurological disorder, developing effective prevention strategies is of great significance. Our MR findings suggest that gout may represent a potentially modifiable risk factor for epilepsy, warranting further clinical investigation.

Data Availability Statement

The datasets supporting the findings of the current study were already openly available when the research project commenced. The GWAS summary statistics for epilepsy, gout, and UA were obtained from the ILAE consortium, the FinnGen consortium, and the BBJ, respectively. The curated instrumental variable datasets and analysis

scripts generated during this study are openly available in the Zenodo repository at <https://doi.org/10.5281/zenodo.18298108>.

Consent for publication of personal information

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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Diet and nutrition in chronic heart failure: A scientific statement of the Polish Society of Dietetics

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Abstract

Poor nutritional status and suboptimal dietary patterns are strongly associated with adverse outcomes in patients with chronic heart failure (CHF), including increased mortality, reduced functional capacity, and a higher risk of hospitalization. This scientific statement from the Polish Society of Dietetics summarizes the current evidence and provides clinically oriented guidance on the role of nutrition in the management of CHF. It outlines the key components of nutritional care across the spectrum of CHF, including screening, assessment, dietary strategies, and supplementation. The main features of evidence-based nutritional management in CHF include: 1) routine nutritional screening using validated tools and structured assessment incorporating the GLIM criteria; 2) identification of malnutrition, cachexia, sarcopenia, and sarcopenic obesity; 3) individualized dietary management tailored to the HF phenotype and clinical status; 4) prioritization of dietary patterns rich in plant-based and minimally processed foods, with the Mediterranean diet as the dietary model supported by the strongest evidence; 5) avoidance of excessive sodium intake and unnecessary fluid restriction in stable patients; 6) ensuring adequate energy intake and protein intake to preserve lean body mass; and 7) targeted, rather than routine, use of micronutrient supplementation, with particular consideration of iron deficiency.

Key words: dietitians, dietary patterns, heart failure, nutritional status, nutrition therapy

Conflict of interest

M.C. reports receiving honoraria for a single educational lecture from NORSAN. This relationship had no influence on the preparation, interpretation, or content of the present manuscript. S.W. reports receiving honoraria from Amgen for educational activities related to post-myocardial infarction care, including hypercholesterolemia, cardiovascular risk reduction, lifestyle modification, and dietary recommendations. This relationship had no influence on the preparation, interpretation, or content of the present manuscript. K.W. reports receiving honoraria from Polpharma, Zentiva, and Nutricia for educational activities related to lifestyle modification in hypercholesterolemia, cardiovascular risk reduction, and the development of dietary recommendations. This relationship had no influence on the preparation, interpretation, or content of the present manuscript. The remaining authors declare no competing interests.

Highlights

- Nutritional status is a key determinant of prognosis in chronic heart failure, influencing mortality, hospitalization risk, and functional capacity.
- Routine nutritional screening and assessment using validated tools and Global Leadership Initiative on Malnutrition (GLIM) criteria are essential components of heart failure care.
- The Mediterranean diet and other plant-rich, minimally processed dietary patterns are the most evidence-supported nutritional approaches for chronic heart failure.
- Management should prioritize adequate energy and protein intake, while micronutrient supplementation should be targeted, particularly in patients with iron deficiency.

Introduction

Heart failure (HF) is a complex clinical syndrome affecting an estimated 64 million people worldwide and representing one of the leading causes of hospitalization and mortality across all age groups.¹ Despite significant advances in pharmacological and device-based therapies, prognosis in chronic HF (CHF) remains poor, with 5-year mortality rates exceeding 50%.² Alongside established risk factors, diet and nutritional status play a pivotal yet underrecognized role in the clinical course of CHF, influencing disease progression, functional capacity, quality of life, and clinical outcomes across the full spectrum of HF phenotypes.^{2,3} CHF is additionally characterized by a high burden of multimorbidity, frailty, inflammation, and metabolic disturbances, all of which may substantially influence nutritional status and clinical management.² No dedicated evidence-based nutritional guidance for patients with HF has been developed to date by a dietetic professional society in Poland or Central Europe.

This scientific statement from the Polish Society of Dietetics (PTD) aims to bridge this gap by synthesizing the current evidence on diet and nutrition across the full spectrum of CHF management, from nutritional screening and assessment to dietary strategies, supplementation, and practical clinical implementation. For each domain, we interpret the available evidence from observational studies, randomized controlled trials (RCTs), meta-analyses, clinical guidelines, and consensus statements from leading international cardiology societies, with particular emphasis on the highest levels of evidence, and highlight areas where further research is needed.

The statement is intended to support dietitians, physicians, nurses, and allied health professionals in delivering evidence-based nutritional care to patients

with CHF. It was developed by a multidisciplinary expert group of 10 professionals with expertise in clinical dietetics, HF nutrition, cardiology, HF nursing, emergency medical services, health promotion, and health education. Collectively, the authors bring extensive clinical and/or research experience in cardiovascular care, nutrition, and public health. The statements presented in this document reflect the authors' expert synthesis and interpretation of the currently available evidence and were refined through iterative discussion and consensus. Given the nature of this scientific statement, no formal grading system for evidence quality or recommendation strength was applied.

Nutrition disorders in chronic heart failure

Statement

STATEMENT 1: Routine nutritional screening using validated tools appropriate for the clinical setting, such as MUST or MNA-SE, is considered an essential component of care for patients with CHF. Positive screening findings warrant a comprehensive nutritional assessment incorporating the GLIM criteria.

STATEMENT 2: In patients with CHF and overweight or obesity, preserving lean body mass and preventing unintentional weight loss are recognized as key priorities in nutritional management, with attention to maintaining adequate nutritional intake throughout the disease course.

Nutrition disorders in HF exist along a complex continuum ranging from protein-energy malnutrition

to cardiac cachexia and sarcopenic obesity.⁴ Malnutrition, diagnosed according to the Global Leadership Initiative on Malnutrition (GLIM) criteria, affects 20–60% of hospitalized patients and is an independent predictor of mortality and prolonged hospital stay.^{4,5} In patients with HF and preserved ejection fraction (HFpEF), the prevalence of malnutrition is estimated at 40–63%, supporting the need for nutritional screening regardless of body mass index (BMI).⁴ Cardiac cachexia represents a multi-organ catabolic syndrome characterized by unintentional weight loss and muscle wasting.⁶ A 2025 meta-analysis involving 3,821 patients revealed that cachexia increases the risk of all-cause mortality by 59% (HR 1.59; 95% CI: 1.34–1.89) and major adverse cardiovascular events (MACE) by 2.41-fold. The pathophysiology of this condition is driven by chronic low-grade inflammation (e.g., elevated tumor necrosis factor alpha (TNF- α) and interleukin (IL)-6) and anabolic resistance.⁶ Additional contributing mechanisms include neurohormonal activation, reduced anabolic signaling, mitochondrial dysfunction, impaired gastrointestinal absorption, and decreased dietary intake, all of which promote progressive muscle wasting and negative energy balance in advanced CHF.^{1,6,7} Commonly used nutritional screening tools in CHF include the Malnutrition Universal Screening Tool (MUST), particularly in the outpatient setting, and the Mini Nutritional Assessment (MNA) or its short form (MNA-SF) in older or frail patients. Comprehensive nutritional assessment may be further supported by the Global Leadership Initiative on Malnutrition (GLIM) framework, which combines phenotypic and etiologic criteria. A detailed discussion of nutritional assessment methods is provided in the “Nutritional assessment in CHF” section.

Sarcopenia (the progressive decline in muscle mass, strength, and function) affects approx. 34% of the HF population.⁸ A 2025 network meta-analysis identified the Short Physical Performance Battery (SPPB) score as a strong predictor of adverse outcomes, although estimates varied substantially across studies and the certainty of the evidence was low.⁸ These muscle-related disorders often lead to frailty, which more than doubles the risk of cardiovascular death.⁸ Conversely, the “obesity paradox” has been described in HF, with observational data suggesting better short- to medium-term survival in patients with a BMI of 25–35 kg/m²; however, this phenomenon is increasingly attributed to residual confounding and the limitations of BMI-based classification rather than a true protective effect.⁹ Analyses incorporating more precise anthropometric indices, such as waist-to-height ratio and measures of central adiposity, demonstrate a consistent association between excess adiposity and adverse clinical outcomes, suggesting that the apparent protective effect of a higher BMI is largely explained by methodological limitations and the inability of BMI to accurately reflect body composition.^{10,11}

However, sarcopenic obesity occurs in 20–30% of patients with overweight, in whom excess adiposity may mask significant muscle deficits.¹² Sarcopenic obesity is associated with markedly reduced functional capacity and higher mortality compared with obesity without muscle loss. Diagnostic strategies must therefore prioritize functional testing and body composition analysis over BMI alone.¹² Taken together, nutrition disorders in HF represent a dynamic and overlapping spectrum requiring comprehensive assessment beyond BMI alone, including attention to modifiable factors such as dietary intake. Reduced appetite and inadequate oral intake are frequently observed in patients with CHF, particularly in older adults, advanced stages of disease, and periods of clinical deterioration.^{3,13} Fatigue, dyspnea, gastrointestinal congestion, and treatment burden may further contribute to reduced dietary intake and progressive loss of lean body mass.^{3,7}

In this context, adequate protein intake appears to be an important determinant of clinical outcomes in patients with HF, particularly in the setting of malnutrition, anabolic resistance, and sarcopenia. Observational data from large HF cohorts indicate that lower protein intake is independently associated with increased all-cause mortality (HR approx. 1.5–2.0 across intake quartiles) and a worse clinical profile, including a greater burden of congestion and higher levels of disease severity biomarkers.¹⁴ Randomized evidence on high-protein interventions remains limited and methodologically heterogeneous. A recent meta-analysis of 15 RCTs ($n = 744$) demonstrated a modest improvement in functional capacity (6-min walk distance [6MWD], mean difference 35 m; 95% CI: 15.93–54.58), without consistent effects on lean body mass, muscle strength, or renal function, and with very low certainty of the evidence due to a high risk of bias and imprecision.¹⁵ An overview of meta-analyses, including 441 studies, demonstrated that protein supplementation does not improve muscle outcomes in the general older population but may confer modest benefits in individuals with chronic conditions, particularly when combined with exercise.¹⁶

These findings are consistent with a systematic review of high-protein dietary interventions, which reported modest improvements in functional outcomes and selected clinical parameters, particularly in malnourished or recently hospitalized patients, but also emphasized substantial heterogeneity across studies in intervention design, patient populations, and outcome measures, thereby limiting the ability to draw firm clinical conclusions.¹⁷ Collectively, the current evidence supports the clinical importance of avoiding inadequate protein intake rather than confirming the efficacy of high-protein dietary strategies. Robust, adequately powered RCTs evaluating clinically meaningful endpoints (mortality, hospitalization, and muscle mass) are lacking.

Nutritional assessment in CHF

STATEMENT 3: Body composition assessment using BIA may provide useful adjunctive information to clinical evaluation in patients with CHF, particularly when interpreted in the context of fluid status and diuretic therapy.

STATEMENT 4: Regular reassessment of nutritional status, particularly following significant changes in clinical condition or body weight, is considered an important component of longitudinal CHF care. Nutritional interventions are best implemented within a multidisciplinary framework involving the treating physician and a registered dietitian.

Nutritional disorders in CHF are frequently underdiagnosed, in part because standard anthropometric measures, such as body mass index, may substantially underestimate nutritional risk when malnutrition, sarcopenia, obesity, or fluid retention coexist, which is common in this population.^{18–20}

Screening tools

There is no single nutritional screening tool specific to HF. Therefore, the choice of screening tool depends on the clinical setting and patient profile. The MUST is practical in outpatient and community care; the Nutritional Risk Screening 2002 (NRS-2002) is more suitable for hospitalized patients, whereas the Mini Nutritional Assessment (MNA) or its short form (MNA-SF) may be especially useful in older and frail individuals with CHF.²¹ In this context, the GLIM framework appears particularly useful because it combines phenotypic criteria, such as weight loss, low BMI, or reduced muscle mass, with etiologic criteria, including reduced intake or absorption and disease burden or inflammation.²² Available evidence suggests that the MNA has good prognostic performance in HF, whereas the MNA-SF is a more practical option.²¹ Laboratory-based scores, such as the Controlling Nutritional Status (CONUT) score (incorporating serum albumin, total cholesterol, and lymphocyte count) or the Geriatric Nutritional Risk Index (GNRI) (based on serum albumin and body weight), may provide additional prognostic information. However, in patients with CHF, these scores should be interpreted with caution because their components may be influenced by inflammation, statin therapy, and fluid retention.²³ In addition, reduced appetite and lower oral intake are frequently observed in nutritionally vulnerable patients with CHF and may contribute to progressive nutritional deterioration, particularly in advanced stages of the disease.¹³

Body composition

Body composition assessment is an important complement to nutritional screening tools because CHF frequently distorts the interpretation of standard

anthropometric measures. Bioelectrical impedance analysis (BIA) is the most practical technique for bedside and outpatient use because it enables repeated assessment of fat mass, fat-free mass, total body water, and extracellular fluid. BIA is a valid adjunctive method for assessing nutritional status because it provides objective information on body composition and hydration, while phase angle may offer additional insight into nutritional status.²⁴ Nevertheless, interpretation of BIA requires consideration of congestion, diuretic therapy, and device-specific limitations. Although currently available evidence suggests a low risk of clinically relevant electromagnetic interference between contemporary BIA devices and cardiac implantable electronic devices (CIEDs), or of CIED malfunction, interpretation of BIA-derived body composition parameters in patients with CHF may still be influenced by congestion status, fluid shifts, and device-related factors that affect measurement accuracy. These limitations are particularly relevant in ambulatory HF management, where BIA findings should be interpreted within the broader clinical and hydration context.^{25,26} Accurate measurement requires standardized conditions, including adequate hydration status and the absence of significant edema, which may limit its reliability during acute decompensation.²⁴ Dual-energy X-ray absorptiometry (DXA) remains the reference method for assessing lean and fat mass and is especially valuable when sarcopenia or sarcopenic obesity is suspected. However, its limited availability, higher cost, and limited feasibility for serial monitoring in outpatient settings restrict its routine clinical applicability.²⁷ Selected laboratory biomarkers may support, but should not replace, clinical nutritional assessment. Albumin should not be used as a standalone marker of nutritional status because low concentrations often reflect inflammation, hemodilution, or disease severity.²⁸ High-sensitivity C-reactive protein can be useful as an indicator of the inflammatory component of disease-related malnutrition and may support the etiologic component of GLIM-based assessment. Iron status has both diagnostic and therapeutic relevance in HF. Current HF guidelines recommend periodic screening for anemia and iron deficiency using a complete blood count, ferritin, and transferrin saturation.² The most useful approach is to combine structured screening, body composition analysis, and a focused biomarker panel rather than relying on any single parameter.

Dietary strategies in CHF

Core nutritional principles in CHF management

Nutritional management in CHF requires an individualized approach that reflects the marked heterogeneity of the syndrome and the high prevalence of nutrition-related disorders affecting disease trajectory.⁷ Contemporary HF phenotypes, including HF with reduced ejection fraction

(HFrEF) and preserved ejection fraction (HFpEF), differ not only in their underlying pathophysiology but also in metabolic profile and nutritional risk burden.^{2,7} Patients with HFrEF are frequently characterized by a hypercatabolic state driven by systemic inflammation and neurohormonal activation, contributing to malnutrition, cachexia, and progressive loss of lean body mass.¹ In contrast, HFpEF is more commonly associated with obesity, adiposity-related inflammation, and cardiometabolic comorbidities, including insulin resistance, representing a phenotype with distinct nutritional challenges.⁷ In clinical practice, nutritional management in CHF is further complicated by the high prevalence of multimorbidity, including chronic kidney disease, diabetes, frailty, obesity, chronic obstructive pulmonary disease, advanced age, and polypharmacy, all of which may substantially influence nutritional risk, dietary tolerance, and the implementation of nutritional strategies.² Contemporary HF pharmacotherapy may also influence nutritional status, body composition, appetite, hydration, and electrolyte balance.^{2,29} In particular, sodium-glucose cotransporter 2 (SGLT2) inhibitors and incretin-based anti-obesity therapies, which are increasingly used in patients with HFpEF and obesity, may contribute to reduced appetite, weight loss, changes in body composition, and altered nutritional requirements, highlighting the importance of individualized nutritional monitoring during treatment.^{29–31} In patients with coexisting chronic kidney disease (CKD), nutritional management may require additional individualization because of the complexity of balancing cardiac and renal nutritional priorities, including considerations related to protein intake, electrolyte management, and fluid balance, as addressed in dedicated nephrology guidelines.

Weight management

STATEMENT 5: Weight management in patients with CHF requires an individualized approach that takes into account the HF phenotype and overall nutritional status. In patients with obesity and HFpEF, intentional weight reduction may improve functional capacity and symptom burden, whereas in HFrEF, nutritional management primarily focuses on preserving lean body mass and minimizing the risk of malnutrition. Nutritional interventions are best implemented under the supervision of a dietitian experienced in HF care.

Body weight is a key determinant of clinical status and prognosis in patients with CHF, although its role varies across HF phenotypes.^{2,7} In HFpEF, obesity represents a dominant clinical and pathophysiologic feature, contributing to systemic inflammation, reduced exercise capacity, and an adverse cardiometabolic profile.⁷ In this population, intentional weight reduction through caloric restriction and aerobic exercise improves functional capacity, as demonstrated by significant increases in peak oxygen consumption, with additive effects observed when

the interventions are combined.³² These findings are consistent with contemporary American College of Cardiology (ACC) recommendations, which emphasize obesity as a key therapeutic target in HFpEF and support lifestyle-based interventions to improve symptoms and functional status.³⁰ Contemporary obesity-targeted therapies may also influence appetite, body composition, and nutritional requirements in patients with obesity-related HFpEF, particularly in the context of incretin-based therapies.³³

In contrast, in HFrEF, the role of weight management is more complex and requires an individualized approach.^{2,7} Unlike HFpEF, unintentional weight loss in HFrEF is consistently associated with worse clinical outcomes, reflecting the predominance of catabolic processes and elevated malnutrition risk in this phenotype.^{1,3} As discussed in the “Nutrition disorders in chronic heart failure (CHF)” section, the apparent protective effect of higher BMI does not reflect a true biological phenomenon, and phenotype-specific assessment of body composition may provide more clinically relevant information than BMI alone.^{10,11} Evidence regarding weight management in patients with heart failure with mildly reduced ejection fraction (HFmrEF) remains limited, and this phenotype is generally considered to lie on a continuum between HFrEF and HFpEF. Taken together, current evidence supports a phenotype-specific approach to weight management in CHF.

Sodium and fluid intake

STATEMENT 6: In patients with CHF receiving guideline-directed medical therapy, excessive salt intake (>5 g/day [approximately >2 g sodium/day]) is generally discouraged, whereas sodium restriction to <1.5 g/day does not appear to confer additional clinical benefit. In stable patients, a usual fluid intake of 1.5–2.5 L/day, guided by thirst and clinical status, is considered appropriate. More restrictive fluid strategies may be relevant in selected patients with dilutional hyponatremia or acute decompensation. Dietary adjustments require individualization according to clinical status and are best undertaken in collaboration with the treating physician.

Sodium and fluid restriction have long been standard recommendations in the management of HF; however, their evidence base has been substantially challenged by contemporary data. The European Society of Cardiology (ESC) Guidelines advise limiting salt intake to no more than 5 g/day in all patients with HF, while fluid restriction to 1.5–2 L/day is recommended only for selected patients with severe or advanced disease.² However, these recommendations have been substantially revised in the clinical consensus statement of the Heart Failure Association (HFA) of the ESC, which concludes that stringent sodium and fluid restriction are not warranted in most patients with stable CHF receiving guideline-directed medical therapy and that excessive restriction may be harmful.³⁴

Regarding sodium, a systematic review and meta-analysis of RCTs found no significant benefit of sodium restriction on all-cause mortality, cardiovascular mortality, or HF-related hospitalization, with a trend toward increased event rates associated with more restrictive sodium intake in ambulatory patients with CHF.³⁵ These findings were confirmed in a separate meta-analysis conducted by Polish investigators, which similarly demonstrated neutral effects on clinical endpoints in outpatient CHF.³⁶ These findings are consistent with the clinical consensus statement of the Heart Failure Association (HFA), which supports a sodium intake of 1.5–4 g/day in patients with stable CHF receiving guideline-directed medical therapy, with intake up to 5 g/day considered acceptable.³⁴

Regarding fluid intake, evidence from randomized trials is similarly limited. The FRESH-UP trial, the largest RCT to date on this topic, randomized 504 outpatients with CHF to liberal fluid intake versus restriction to 1,500 mL/day. No significant difference in health status (Kansas City Cardiomyopathy Questionnaire – Overall Summary Score (KCCQ-OSS): 74.0 vs 72.2; adjusted mean difference 2.17; 95% confidence interval (95% CI): –0.06 to 4.39; $p = 0.06$) was observed, while thirst distress was significantly lower in the liberal intake group (Thirst Distress Scale for patients with HF (TDS-HF): 16.9 vs 18.6; $p < 0.001$), with no differences in mortality, HF hospitalization, or changes in diuretic therapy.³⁷ These findings are in line with the HFA consensus statement, which supports normal fluid intake of 1.5–2.5 L/day guided by thirst, with fluid restriction considered only in selected patients with dilutional hyponatremia or acute decompensation.³⁴

Dietary patterns

STATEMENT 7: The Mediterranean dietary pattern currently represents the dietary approach with the strongest evidence base in patients with CHF and can be adapted to local food availability and cultural context. The Dietary Approaches to Stop Hypertension (DASH) and plant-based dietary patterns also appear to be reasonable dietary approaches for selected patients. Dietary strategies for CHF require individualization based on HF severity, comorbidities, nutritional status, and patient preferences.

Dietary patterns characterized by high intakes of plant-based foods, whole grains, legumes, nuts, and unsaturated fats, including the Mediterranean diet, the DASH diet, and other plant-based dietary patterns, have been shown to be significantly associated with improved cardiovascular outcomes and a reduced risk of HF.^{38–41} The most consistent and well-documented evidence relates to the Mediterranean diet. A systematic review and meta-analysis of cohort studies including 216,385 participants from European countries who were free of HF at baseline revealed that each 1-point increase in adherence to the Mediterranean

diet was associated with a significantly lower risk of HF (RR = 0.94; 95% CI: 0.912–0.969). The authors concluded that greater adherence to this dietary pattern was associated with a lower risk of HF, particularly among women.³⁸ These findings are consistent with a meta-analysis of observational studies evaluating the Mediterranean and DASH dietary patterns, in which high adherence to the Mediterranean diet was associated with a lower risk of incident HF (odds ratio (OR) = 0.77; 95% CI: 0.63–0.93), whereas high adherence to the DASH diet was associated with a lower risk of HF (OR = 0.83; 95% CI: 0.70–0.98). Importantly, in the same analysis, higher adherence to the Mediterranean diet was also associated with lower all-cause mortality in patients with HF (OR = 0.88; 95% CI: 0.78–0.99), whereas similarly robust data for the DASH diet in patients with established HF were not demonstrated.³⁹ These findings suggest that, although both dietary patterns are beneficial for HF prevention, the association between diet and the clinical course of CHF is best documented for the Mediterranean diet, although the available evidence should still be interpreted with caution.

From a practical perspective, the feasibility of implementing the Mediterranean diet outside its region of origin is also important. Cost-adapted and culturally adapted Mediterranean dietary models should focus not on replicating specific foods typical of the Mediterranean region but rather on preserving the structural characteristics of the dietary pattern: high intakes of vegetables, fruits, legumes, whole grains, nuts, and seeds; a predominance of unsaturated over saturated fats; regular consumption of fish; and reduced intake of red and processed meats, ultra-processed foods, and added sugars. In Central and Eastern European settings, this may involve using locally available seasonal vegetables, legumes, whole grains, nuts, seeds, and accessible sources of unsaturated fats while maintaining overall dietary quality. Such an approach enhances feasibility, acceptability, and long-term adherence to dietary interventions, which is particularly important in patients with chronic disease.^{42,43} Similarly, the DASH dietary pattern, originally developed for blood pressure control, has demonstrated favorable associations with HF risk in observational studies. In a large prospective cohort study, long-term adherence to this dietary pattern was associated with a significantly reduced risk of HF (HR = 0.83–0.85), with additional benefits observed when red and processed meat was replaced by DASH-consistent food components.⁴⁴ However, in patients with established HF, the available evidence is considerably more limited. In one study of patients with HF, better adherence to the DASH diet was associated with lower insulin resistance, which may be relevant in the context of coexisting metabolic disturbances but does not constitute evidence of improved hard HF endpoints.⁴⁵ Therefore, the DASH diet may be considered a favorable cardiometabolic dietary pattern, particularly for patients with HF and hypertension or metabolic disorders; however, its direct impact on the clinical course of CHF has not been well established.

Plant-based dietary patterns, including vegetarian and vegan diets, also represent dietary models with a potentially favorable cardiovascular profile. In population-based cohort studies, a higher intake of plant-based foods and lower consumption of animal products were associated with a reduced risk of cardiovascular morbidity, cardiovascular mortality, and all-cause mortality.⁴⁶ Furthermore, a systematic review and meta-analysis of clinical trials in individuals with cardiovascular disease (CVD) or at high cardiovascular risk demonstrated that vegetarian diets were associated with reductions in low-density lipoprotein cholesterol (LDL-C), glycated hemoglobin (HbA1c), and body weight beyond those achieved with standard care.⁴⁷ However, direct evidence regarding plant-based diets in the treatment of CHF remains very limited. In clinical practice, each of these dietary patterns requires adaptation to the specific context of CHF, and the Mediterranean diet currently has the strongest evidence base in this area. Adherence to this dietary pattern is associated with a lower risk of HF development, and available data also suggest potential benefits in patients with established HF, particularly with respect to all-cause mortality. For the DASH diet and plant-based dietary patterns, including vegetarian and vegan diets, high-quality randomized trials and meta-analyses conducted specifically in populations with CHF are still lacking. Much of the currently available evidence on dietary patterns is derived from cardiovascular prevention cohorts or populations free of HF at baseline, whereas high-quality randomized studies conducted specifically in patients with established CHF remain limited. Therefore, although these dietary patterns appear promising as adjunctive strategies in HF management, their effects should be interpreted with caution, and further research is needed to establish HF-specific recommendations.

Alcohol

STATEMENT 8: Alcohol consumption in patients with CHF is associated with potential adverse cardiovascular and pharmacological effects and does not appear to confer clinical benefit in this population. In patients with alcohol-related cardiomyopathy, complete alcohol abstinence remains a central component of management. Discussion of alcohol-related risks may support individualized counseling within routine CHF care.

Alcohol consumption in patients with established CHF should be approached with particular caution because it is associated with dilated cardiomyopathy, worsening HF, arrhythmias, and adverse interactions with guideline-directed pharmacotherapy.^{2,48,49} Beyond its cardiovascular effects, alcohol is classified as a Group 1 carcinogen by the International Agency for Research on Cancer, and available evidence indicates that no level of alcohol consumption can be considered safe with respect to cancer risk.^{50,51} This is particularly relevant in patients with CHF,

given the high prevalence of comorbidities and polypharmacy in this population. Current cardiovascular guidelines advise against alcohol consumption in patients with cardiomyopathy or established HF, and heavy alcohol consumption is consistently associated with worse cardiovascular outcomes.^{48,52,53}

Energy intake

STATEMENT 9: Estimation of energy requirements in patients with CHF requires an individualized approach based on nutritional status, clinical condition, and body composition. Validated weight-based equations represent the most practical approach in routine care, whereas indirect calorimetry may provide additional value in selected cases where available.

STATEMENT 10: Total energy intake in patients with CHF is aimed at supporting weight stability in clinically stable individuals and nutritional recovery in patients with malnutrition or cachexia. Energy requirements and intake may require reassessment in response to changes in clinical status, nutritional condition, or body weight.

Energy requirements in patients with CHF vary according to nutritional status, disease severity, and clinical setting. Indirect calorimetry provides the most accurate estimate of resting metabolic rate (RMR) and is considered the reference method when available.⁵⁴ If indirect calorimetry is not feasible, RMR may be estimated using 22 kcal/kg of actual body weight in well-nourished patients and 24 kcal/kg in malnourished patients with CHF, with modifications required in advanced disease.^{55,56} The estimated RMR should be multiplied by an appropriate physical activity factor to determine total energy requirements. In stable, euvolemic patients, energy intake should meet requirements for weight maintenance and the prevention of catabolism. In malnourished or cachectic patients, energy intake should be sufficient to support nutritional recovery, whereas in patients with acute decompensated HF, energy intake should be reassessed regularly based on clinical tolerance.

In patients with overweight or obesity, particularly in the HFpEF phenotype, as discussed in the “Weight management” section, energy intake may be temporarily reduced to achieve gradual, intentional weight loss, provided the intervention is implemented in a controlled manner and does not lead to loss of lean body mass. The energy deficit should be moderate and combined with adequate protein intake and, where feasible, physical activity.^{32,57} In patients with coexisting chronic kidney disease, nutritional planning may be further complicated by the need to balance cardiac and renal nutritional priorities, particularly regarding protein intake, electrolyte disturbances, and fluid management, while taking into account dedicated nephrology guidelines in this area.⁵⁸

Diet fortification and oral nutritional support

STATEMENT 11: In patients with CHF who are malnourished or at risk of malnutrition, individualized nutritional support, including diet fortification and oral nutritional supplements (ONS), may improve nutritional status and appear to be associated with favorable clinical outcomes, including reduced mortality and HF-related rehospitalization. Nutritional management of malnutrition in CHF benefits from alignment with established clinical nutrition frameworks, including European Society for Clinical Nutrition and Metabolism (ESPEN) guidance, while taking into account the patient's clinical status, comorbidities, and nutritional needs.

Malnutrition affects approx. 46% of patients with CHF and is independently associated with a more than twofold increase in all-cause mortality.³ In patients with HF who are malnourished or at nutritional risk, early individualized nutritional support is associated with improved clinical outcomes. It has been shown to reduce 30-day mortality from 14.8% to 8.4% compared with a standard hospital diet.⁵⁹ The first step in nutritional intervention is diet fortification, increasing the energy and protein density of regular meals through the addition of protein powders, oils, or between-meal snacks, adapted to the patient's preferences, fluid restrictions, and comorbidities (e.g., renal failure and diabetes). When diet fortification alone is insufficient to meet at least 75% of estimated energy and protein requirements (1.2–1.5 g protein/kg body weight/day), oral nutritional supplements (ONS) should be introduced. High-calorie, high-protein ONS have been shown to increase body weight by a mean of 3.83 kg (95% CI: 0.17–7.50, $p = 0.04$) in malnourished patients with HF and, when combined with individualized dietary counseling, to significantly reduce all-cause mortality and HF-related rehospitalization.¹⁸ In patients whose oral intake remains insufficient despite diet fortification and ONS, escalation to enteral nutrition should be considered. Nutritional status should be reassessed regularly throughout the hospital stay and at discharge. Figure 1 presents the nutritional management algorithm for patients with CHF.

Micronutrients and supplementation

Iron deficiency and iron therapy

STATEMENT 12: Adequate dietary iron intake represents an important component of nutritional management in patients with CHF. In the presence of confirmed iron deficiency, current evidence supports intravenous iron supplementation as the preferred therapeutic approach.

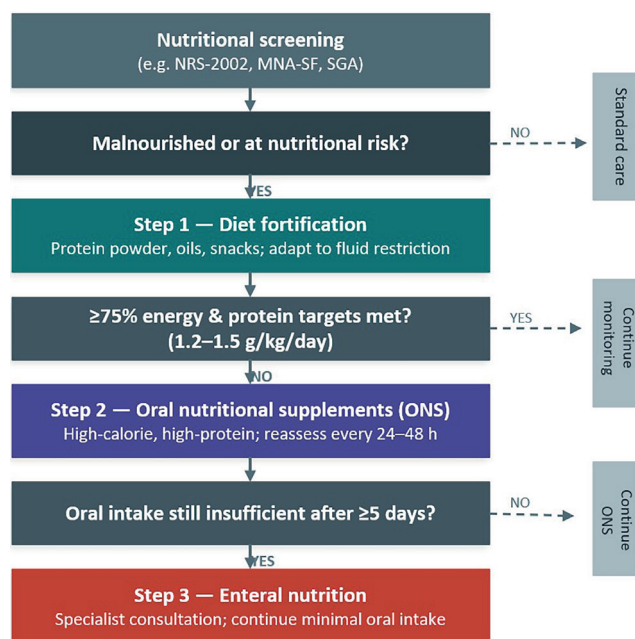


Fig. 1. Nutritional management algorithm for patients with chronic heart failure. Proposed stepwise approach to nutritional management in patients with chronic heart failure (CHF), beginning with routine nutritional screening and progressing through diet fortification, oral nutritional supplementation, and enteral nutrition when oral intake remains insufficient. The algorithm emphasizes regular reassessment of nutritional status, achievement of energy and protein targets, and individualized nutritional support according to clinical status and treatment tolerance

Iron deficiency affects approx. 50% of patients with CHF and is an independent predictor of all-cause mortality, regardless of hemoglobin concentration. Its development is related to both reduced dietary iron intake and impaired intestinal absorption resulting from inflammation-induced elevation of hepcidin levels.⁶⁰ Adequate dietary iron intake remains an important component of the nutritional management of patients with CHF; however, oral iron supplementation does not effectively correct iron deficiency in this population. In the IRONOUT HF trial ($n = 225$), high-dose oral iron supplementation with a polymeric iron complex (150 mg twice daily for 16 weeks) resulted in only a modest 3% increase in transferrin saturation and did not improve exercise capacity or quality of life compared with placebo.⁶¹ A meta-analysis of 5 RCTs ($n = 590$) confirmed that oral iron did not significantly improve transferrin saturation, 6MWD, or all-cause mortality.⁶² A key mechanistic barrier limiting the bioavailability of oral iron in CHF is elevated hepcidin.⁶¹ By contrast, intravenous iron supplementation reduces recurrent HF hospitalizations and cardiovascular mortality. In a meta-analysis of 10 RCTs ($n = 3,373$), a 25% reduction in the composite endpoint was observed (rate ratio = 0.75; 95% CI: 0.61–0.93; $p < 0.01$),⁶³ which was confirmed by an updated Bayesian meta-analysis of 6 studies including 7,175 patients (RR = 0.72; 95% CI: 0.55–0.89 at the 12-month follow-up).⁶⁴

Vitamin D

STATEMENT 13: Current evidence does not support routine vitamin D supplementation as HF-specific therapy in patients with CHF. Correction of confirmed vitamin D deficiency may nevertheless be appropriate according to general clinical indications. Further well-designed randomized trials are needed, particularly those accounting for HF phenotype, baseline 25(OH)D concentration, and clinically relevant outcomes.

Vitamin D is an important nutrient involved in multiple pathophysiological pathways relevant to HF. In patients with HF, low 25(OH)D concentrations are frequently associated with a worse prognosis. A meta-analysis showed that low circulating 25(OH)D concentrations may be an independent risk factor for all-cause mortality in patients with HF.⁶⁵ However, it should be emphasized that this observational association does not consistently translate into benefit from supplementation, and randomized evidence remains inconsistent. In a meta-analysis of 7 RCTs, vitamin D supplementation had no significant effect on LVEF, exercise capacity, or natriuretic peptide concentrations.⁶⁶ By contrast, a more recent meta-analysis including 18 RCTs ($n = 2,182$) reported improvements in selected surrogate markers, such as left ventricular ejection fraction (LVEF), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and indices of left ventricular remodeling.⁶⁷ These findings should, however, be interpreted with caution because of substantial heterogeneity across studies, differences in dosing regimens, treatment duration, and population characteristics. Overall, the current evidence does not provide a sufficiently strong basis for the routine use of vitamin D as an HF-specific therapy, although correction of confirmed deficiency remains appropriate according to general clinical indications.

Thiamine and B-group vitamins

STATEMENT 14: Current evidence does not support routine supplementation with thiamine or other B-group vitamins as HF-specific therapy in patients with CHF. Correction of confirmed deficiency may nevertheless be appropriate according to general clinical indications.

Among the B vitamins, thiamine has received the greatest attention in HF because of its role in energy metabolism and the potential impact of deficiency on cardiac function. In patients with HF, thiamine deficiency appears to be more common than in the general population, which may reflect poorer nutritional status, more advanced disease, and polypharmacy.⁶⁸ However, interventional data remain inconsistent. Meta-analyses of randomized trials have not demonstrated a significant effect of thiamine supplementation on LVEF, NT-proBNP, exercise capacity, or New

York Heart Association (NYHA) functional class, while indicating that its principal effect is the correction of thiamine deficiency.^{69,70} Evidence regarding other B vitamins is even more limited, and the small sample sizes, heterogeneity of the available studies, and lack of hard clinical endpoints do not currently support routine supplementation as an HF-specific therapy.

Potassium and magnesium

STATEMENT 15: Dietary potassium and magnesium intake in patients with CHF requires individual assessment in the context of serum electrolyte concentrations, renal function, and contemporary pharmacotherapy. Potassium-containing salt substitutes may be a clinically relevant source of excess potassium intake in patients with hyperkalemia or those receiving Renin-Angiotensin-Aldosterone System inhibitors (RAASI) therapy. Current evidence does not support routine magnesium supplementation in the absence of symptomatic or documented deficiency.

Electrolyte disturbances are common in CHF and are closely linked to pharmacological treatment. Loop diuretics and thiazides promote urinary losses of potassium and magnesium, increasing the risk of hypokalemia and hypomagnesemia, whereas angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), and mineralocorticoid receptor antagonists may cause potassium retention and hyperkalemia.^{2,71} Both hypokalemia and hyperkalemia are independently associated with increased mortality in patients with CVD, including CHF, with a U-shaped relationship and the lowest risk observed at a serum potassium concentration of approx. 4.2 mmol/L. A meta-analysis of 31 cohort studies ($n = 227,645$) demonstrated that hyperkalemia was associated with a nearly 3-fold increased risk of in-hospital mortality (RR = 2.78; 95% CI: 1.92–4.03) and a 1.3-fold increased risk of long-term mortality (RR = 1.33; 95% CI: 1.19–1.48), with similar associations observed for hypokalemia.⁷² In patients receiving renin–angiotensin–aldosterone system inhibitor (RAASi) therapy, hyperkalemia represents a clinically significant risk, and potassium-containing salt substitutes have been identified as an underrecognized dietary source of excess potassium intake in this population.⁷¹

Excessive dietary potassium restriction may further complicate the optimization of guideline-directed HF pharmacotherapy, highlighting the need for individualized dietary assessment and biochemical monitoring.^{2,71} A detailed dietary history, including the use of supplements and salt substitutes, is therefore essential. Robust interventional evidence for dietary potassium management specifically in CHF remains limited, and no adequately powered RCTs have been completed to date. Regarding magnesium, diuretic-induced urinary losses are a common cause of hypomagnesemia in CHF. However, a prespecified

analysis of the GALACTIC-HF trial (n = 6,147 outpatients with HFrEF) demonstrated that hypomagnesemia was not associated with a higher risk of the primary composite outcome or sudden death compared with normal magnesium concentrations, whereas hypermagnesemia was associated with significantly worse outcomes.⁷³ These findings do not support routine correction of hypomagnesemia in ambulatory patients with HFrEF.

Omega-3 fatty acids

STATEMENT 16: Current evidence does not support routine omega-3 supplementation as HF-specific therapy in patients with CHF. Nevertheless, adequate dietary intake of omega-3 fatty acids represents a relevant component of overall dietary quality, while supplementation may be appropriate in selected individuals following individualized clinical assessment.

Omega-3 fatty acids have been evaluated as a potential adjunctive therapy in HF, but the available data remain inconclusive. In a meta-analysis assessing the effects of omega-3 supplementation on inflammatory markers, supplementation reduced TNF- α and IL-6 concentrations but had no significant effect on C-reactive protein (CRP).⁷⁴ At the same time, a meta-analysis of 12 trials including 81,364 participants found no reduction in the risk of first HF hospitalization or cardiovascular mortality, suggesting only a possible reduction in recurrent HF hospitalizations.⁷⁵ A more recent network meta-analysis of randomized trials suggested that long-term supplementation with higher doses of n-3 polyunsaturated fatty acids (2,000–4,000 mg/day) may improve selected surrogate outcomes, such as LVEF and peak oxygen uptake (VO₂peak), without a demonstrated effect on all-cause mortality; similar effects were not observed with lower doses or shorter treatment durations.⁷⁶ These findings should be interpreted with caution because of heterogeneity among the study populations, differences in dose, formulation, and treatment duration, and the limited number of long-term studies. Notably, omega-3 fatty acid supplementation has been associated with a modest, dose-dependent increase in the risk of atrial fibrillation in cardiovascular populations, which may be relevant when considering its use in patients with HF.^{77,78}

Coenzyme Q10

STATEMENT 17: Coenzyme Q10 supplementation appears to represent a potentially beneficial adjunctive strategy in selected patients with symptomatic HF, although the current evidence base remains limited and heterogeneous. Available data do not currently support routine supplementation in all patients with CHF. Decisions regarding CoQ10 use require individualized clinical assessment.

Coenzyme Q10 (CoQ10) plays an important role in mitochondrial energy metabolism and has antioxidant properties; it has therefore been investigated as a potential adjunctive therapy in HF. The most compelling data come from the Q-SYMBIO trial, in which 2 years of CoQ10 supplementation at a dose of 100 mg 3 times daily was associated with reductions in major adverse cardiovascular events, all-cause mortality, and HF hospitalization, despite no significant benefit in short-term endpoints.⁷⁹ A Cochrane systematic review concluded that CoQ10 probably reduces all-cause mortality and HF-related hospitalization, although the certainty of the evidence for functional outcomes remains very low.⁸⁰ A more recent meta-analysis of RCTs confirmed beneficial effects on mortality, hospitalization, B-type natriuretic peptide (BNP) concentrations, 6MWD, and left ventricular ejection fraction (LVEF). However, these findings should be interpreted with caution because of the small sample sizes and heterogeneity among the included studies, as well as important methodological limitations.⁸¹

Zinc and selenium

STATEMENT 18: Current evidence does not support routine zinc or selenium supplementation in patients with CHF without documented deficiency. Assessment of trace element status may nevertheless provide additional clinical information in patients with severe malnutrition, significant gastrointestinal dysfunction, or prolonged high-dose diuretic therapy.

Zinc deficiency is prevalent in patients with CHF and may result from reduced dietary intake, impaired gastrointestinal absorption due to bowel edema, increased intestinal losses, and diuretic-induced urinary excretion.⁸² A multicenter retrospective cohort study of 8,290 patients with HF demonstrated that zinc deficiency was associated with significantly higher risks of all-cause mortality (HR = 1.46; 95% CI: 1.26–1.69), major adverse cardiovascular events, and major adverse kidney events during 1 year of follow-up compared with patients with normal zinc levels.⁸³ Evidence supporting routine zinc supplementation in CHF remains limited, and no adequately powered RCTs have been completed to date. Selenium concentrations are generally lower in patients with CHF than in healthy individuals, and low selenium levels have been associated with impaired cardiac function and systemic inflammation.⁸⁴ However, current evidence does not support routine selenium supplementation in CHF. Selenium deficiency (serum selenium <70 μ g/L) was present in 20% of patients with HF and was independently associated with a 52% higher risk of all-cause mortality (HR = 1.52; 95% CI: 1.26–1.86).⁸⁵ However, interventional evidence from adequately powered RCTs in patients with CHF remains lacking. Overall, despite promising findings for selected nutritional supplements, HF-specific randomized evidence remains limited for many supplementation strategies, and their routine use cannot currently be broadly recommended.

Practical nutritional management

STATEMENT 19: Integration of a registered dietitian within the multidisciplinary CHF care team represents an important component of comprehensive nutritional management. Dietary counseling benefits from incorporating evidence-based behavior change strategies while taking into account the patient's clinical status, health literacy, and socioeconomic context. Socioeconomic barriers that affect access to healthy dietary patterns are increasingly recognized as clinically relevant determinants of nutritional care for patients with CHF.

Effective nutritional management in CHF extends beyond prescribing dietary recommendations and requires a structured, patient-centered approach embedded within multidisciplinary care (Fig. 2). The inclusion of a registered dietitian in multidisciplinary HF care may facilitate nutritional assessment, individualized counseling, and longitudinal monitoring. Evidence from a meta-analysis of 30 RCTs ($n = 7,950$) demonstrates that nurse-coordinated multidisciplinary care significantly reduces all-cause mortality ($RR = 0.80$; 95% CI: 0.72–0.88; $p < 0.001$), HF-related hospitalization ($RR = 0.56$; 95% CI: 0.45–0.71), and all-cause hospitalization ($RR = 0.78$; 95% CI: 0.70–0.87) compared with usual care.⁸⁶ Dietary support delivered within such a framework is therefore not an add-on but an integral component of evidence-based CHF management. Regular follow-up and reassessment of nutritional status have been associated with improved dietary adherence and clinical outcomes in patients with CVD, although robust evidence, particularly in patients with CHF, remains limited.⁸⁷

Patient education and adherence to dietary recommendations remain critical challenges in CHF. Adherence to lifestyle and dietary recommendations is consistently reported as suboptimal; however, evidence linking poor adherence directly to symptom burden and HF-related hospitalizations remains limited and is largely observational. The Heart Failure Association (HFA) of the European Society of Cardiology (ESC) recommends that self-care education, including nutritional counseling, incorporate structured behavioral support, address individual barriers to adherence, and actively involve family members or caregivers.⁸⁷ Effective strategies include motivational interviewing, shared decision-making, and self-monitoring tools tailored to the patient's health literacy, cognitive status, and social context.

Socioeconomic factors represent a frequently overlooked yet clinically relevant barrier to optimal nutritional care in CHF. The financial burden of HF, including medication costs and frequent healthcare encounters, may significantly limit patients' ability to follow recommended dietary patterns. Food insecurity is associated with higher cardiovascular and all-cause mortality, independent of other socioeconomic and lifestyle factors.⁸⁸ These disparities represent important barriers to the delivery of effective nutritional care.

Future research directions

Despite growing interest in nutritional interventions in CHF, the evidence base remains limited and methodologically heterogeneous. Several priority areas for future research have been identified. Current evidence does not allow for clear differentiation of dietary recommendations between HFrEF and HFpEF, although these phenotypes differ substantially in their metabolic profiles and nutritional risk burden. Adequately powered RCTs comparing phenotype-specific nutritional strategies are therefore needed. The role of the gut microbiome in nutritional status, systemic inflammation, and disease progression in CHF also represents an emerging and underexplored area, with interventional data in patients with CHF currently lacking. Sarcopenic obesity, the coexistence of excess adiposity and reduced lean mass, is increasingly recognized in CHF, particularly in HFpEF; however, no dedicated nutritional intervention trials have addressed this phenotype. Validated diagnostic criteria and targeted dietary strategies for this population are urgently needed. Perhaps most importantly, there are no adequately powered randomized trials evaluating the impact of dietitian-led nutritional care versus standard care on hard clinical endpoints, including mortality, HF hospitalization, and quality of life, in ambulatory patients with CHF. Such trials should reflect real-world outpatient settings and include standardized nutritional assessments and intervention protocols. Finally, nutritional research in CHF has historically underrepresented older adults, women, and patients from lower socioeconomic backgrounds. Future studies should prioritize these populations to ensure the generalizability and equity of nutritional recommendations.

Conclusions

Nutrition is a fundamental but still underutilized component of CHF care. Patients with CHF require routine nutritional screening, assessment of body composition and muscle function, and early identification of malnutrition, cachexia, sarcopenia, and sarcopenic obesity. Nutritional management should be individualized according to HF phenotype, clinical status, comorbidities, renal function, socioeconomic context, and patient preferences. The Mediterranean diet currently represents the dietary pattern supported by the strongest evidence base, whereas the DASH diet and plant-based dietary patterns may be appropriate alternatives for selected patients. Current evidence does not support excessive sodium or fluid restriction in patients with stable CHF, and nutritional supplementation should be targeted rather than routine, with intravenous iron remaining the treatment of choice for confirmed iron deficiency. Integration of a registered dietitian into multidisciplinary CHF care may support

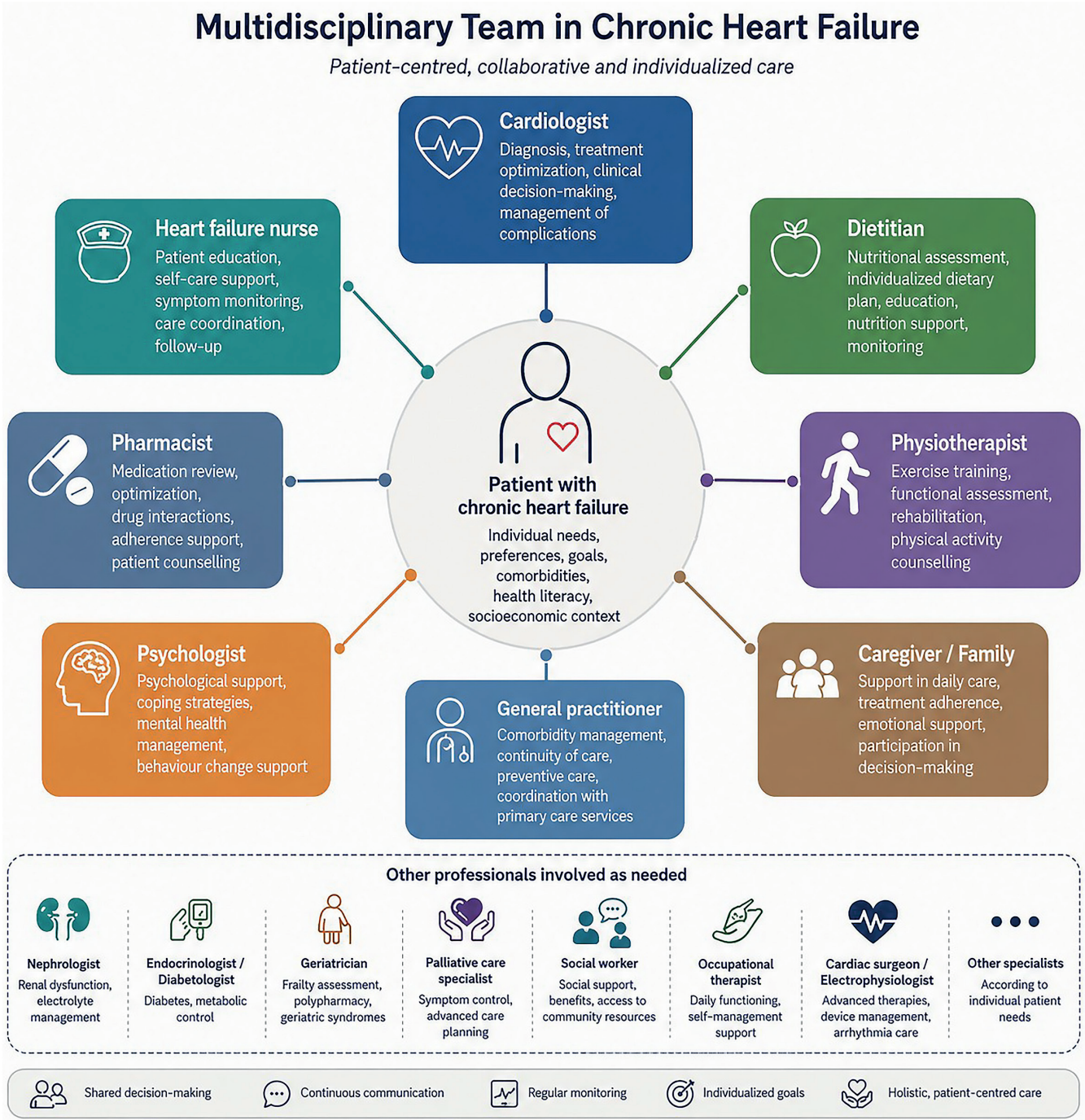


Fig. 2. Multidisciplinary team approach to chronic heart failure (CHF). Patient-centered, collaborative care model illustrating the roles of key healthcare professionals involved in the management of chronic heart failure, including cardiologists, dietitians, nurses, physical therapists, pharmacists, psychologists, caregivers, and primary care physicians. The model emphasizes individualized care based on patient needs, comorbidities, nutritional status, health literacy, and socioeconomic context, supported by shared decision-making, continuous monitoring, coordinated care pathways, and long-term follow-up. The figure also highlights the contribution of extended multidisciplinary support, including nephrology, diabetology, geriatrics, palliative care, social care, occupational therapy, and advanced HF specialist services, according to individual patient complexity and clinical needs

nutritional assessment, counseling, implementation, and follow-up. Future studies should evaluate phenotype-specific, dietitian-led nutritional interventions with respect to hard clinical outcomes, functional capacity, quality of life, and body composition.

Use of AI and AI-assisted technologies

OpenAI’s ChatGPT was used for language editing and proofreading to ensure the final manuscript’s clarity, coherence, and linguistic accuracy.

Data Availability Statement

No new datasets were generated or analyzed in the preparation of this scientific statement. All evidence discussed is derived from the sources listed in the References section

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Gut microbiome in type 2 diabetes mellitus: A literature review

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Abstract

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder influenced by genetic, environmental, and lifestyle factors. Emerging evidence highlights the crucial role of the gut microbiota (GM) in the pathophysiology of T2DM, with alterations in microbial diversity and composition contributing to insulin resistance, systemic inflammation, and metabolic dysregulation. This review explores the current literature on the role of the GM, its metabolites, including short-chain fatty acids (SCFAs), bile acids (BAs), and branched-chain amino acids (BCAAs), as well as the impact of diet and body mass index (BMI) on the progression of T2DM. We examine how dietary patterns, including fiber intake, fat composition, and the use of prebiotics and probiotics, modulate the GM. Furthermore, we analyze evidence regarding the influence of the microbiome on host metabolism, gut permeability, and the pathophysiology of T2DM. Understanding the dynamic interactions among the GM, microbial metabolites, diet, and BMI may provide promising avenues for the development of personalized therapeutic strategies targeting microbiome-mediated mechanisms in the prevention and management of T2DM. Such approaches may facilitate more effective patient-centered interventions.

Key words: probiotics, diet, type 2 diabetes mellitus, body mass index, gut microbiota

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Highlights

- Altered gut microbial composition contributes to insulin resistance, inflammation, and metabolic dysregulation in type 2 diabetes mellitus (T2DM).
- Short-chain fatty acids, bile acids (BAs), and branched-chain amino acids influence host metabolism and may contribute to disease progression.
- Dietary patterns, including fiber intake, fat composition, and probiotic use, modulate gut microbiome diversity and metabolic health.
- Understanding gut microbiota (GM)–diet–body mass index (BMI) interactions may support the development of precision interventions for the prevention and treatment of T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a prevalent chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance (IR) and/or inadequate insulin secretion.¹ The global prevalence of T2DM has increased rapidly, with approx. 537 million adults affected in 2021. Projections suggest that this number will rise to approx. 783 million by 2045.² Type 2 diabetes mellitus is closely associated with obesity and a sedentary lifestyle and is frequently accompanied by chronic low-grade inflammation and metabolic dysregulation.³ In recent years, accumulating evidence has implicated the gut microbiota (GM) – a complex community of trillions of microorganisms residing in the gastrointestinal tract – in both the pathogenesis and progression of T2DM.⁴ The human GM plays a critical role in nutrient digestion, energy harvest, immune modulation, and the production of bioactive metabolites, including short-chain fatty acids (SCFAs), bile acids (BAs), branched-chain amino acids (BCAAs), and trimethylamine N-oxide (TMAO). Disruption of its composition, referred to as dysbiosis, may adversely affect host metabolism.² Studies have shown that individuals with T2DM often exhibit a distinct gut microbial profile compared with non-diabetic individuals.⁵

These alterations include changes in microbial diversity and shifts in the relative abundance of specific bacterial taxa, some of which have been associated with IR, impaired glucose metabolism, and increased gut permeability.⁶ These observations raise the question of whether gut dysbiosis contributes to the development or progression of T2DM. Conversely, T2DM itself, together with its common comorbidities, such as obesity, may also drive alterations in GM composition, suggesting a bidirectional relationship. Understanding this reciprocal interaction is crucial, as it may pave the way for novel therapeutic strategies targeting the GM. Dietary interventions, including high-fiber diets, the Mediterranean diet, ketogenic diets, probiotics, prebiotics, and even fecal microbiota transplantation, are currently being explored as approaches to modulate the GM and potentially improve metabolic outcomes in T2DM.⁶

This paper presents a literature review of recent evidence on GM alterations associated with T2DM, focusing on meta-analyses and systematic reviews published over the past 2 years. We systematically mapped current findings across microbial diversity and taxonomic composition, GM-derived metabolites, dietary influences, gut permeability, and microbiota-targeted therapeutic strategies.

Objectives

The objective of this review was to evaluate recent scientific evidence regarding alterations in GM composition and diversity associated with obesity and T2DM, as well as to analyze the role of microbial metabolites, including SCFAs, BAs, and BCAAs, in the pathophysiology of T2DM. The review further examines the impact of dietary patterns (such as fiber intake, fat composition, and the use of prebiotics and probiotics) on GM in individuals with T2DM, as well as the association between GM, BMI, and metabolic outcomes. In addition, it assesses the relationships among gut permeability, systemic inflammation, and IR as influenced by the GM in T2DM, and identifies potential microbiota-targeted therapeutic strategies, including dietary modulation, probiotics, and fecal microbiota transplantation, for the prevention and management of T2DM.

Methodology

The literature search was conducted using 2 electronic databases, PubMed and Embase, covering the period from January 1, 2022, to February 25, 2025. The search strategy included the following keywords: “type 2 diabetes and gut microbiota” and “changes in gut microbiota and type 2 diabetes.” The initial database search identified 2,087 records. Duplicate publications were removed prior to screening. Four authors (K.W., A.P., J.K., and R.S.) independently screened titles and abstracts to identify the most relevant studies. A total of 114 publications were selected based on the predefined inclusion criteria.

Diversity of gut microbiome

Microbial diversity can be assessed using alpha and beta diversity metrics. Alpha diversity reflects the richness and abundance of microbial species within a specific microbial community, whereas beta diversity measures the degree of variation between GM communities.⁵

Alpha diversity

A recent systematic review of cross-sectional studies involving 65,754 individuals, including 1,800 with T2DM and 2,770 with prediabetes, reported that alpha diversity was lower in T2DM and prediabetic cohorts in 86% of the included studies compared with healthy controls.⁶ These findings suggest a reduction in microbial richness and/or evenness associated with the diabetic state. However, a 2025 meta-analysis of 13 case-control studies found no consistent differences in alpha diversity between patients with T2DM and healthy control groups, highlighting substantial inter-study heterogeneity ($p > 0.05$; $I^2 > 75\%$).⁷ Similarly, a meta-analysis by Pinart et al. reported lower alpha diversity in obese individuals in a substantial proportion of the included studies; however, analysis of the Shannon index revealed no significant differences (mean difference (MD)= 0.06, $p > 0.05$, $I^2 = 81\%$).⁸ These inconsistencies likely reflect differences in BMI, medication use, dietary patterns, geographic location, and sequencing methodologies across studies.^{5,9}

Beta diversity

Nevertheless, numerous studies consistently indicate that beta diversity differs between individuals with and without diabetes. Thus, even when changes in overall microbial diversity are modest or inconsistent, shifts in the relative abundance of specific microbial taxa are frequently observed in diabetes, suggesting that the GM of individuals with diabetes differs from that of healthy individuals.⁵ Nevertheless, numerous studies consistently indicate that beta diversity differs between individuals with and without diabetes. Thus, even when changes in overall microbial diversity are modest or inconsistent, shifts in the relative abundance of specific microbial taxa are frequently observed in diabetes, suggesting that the GM of individuals with diabetes differs from that of healthy individuals.⁵ Slouha et al.¹ analyzed studies involving populations of diverse ethnic backgrounds and observed similar outcomes. These findings are further supported by other authors.^{10–13} Furthermore, Xu et al. demonstrated that dietary patterns, including the Mediterranean diet and ketogenic diets, significantly influence the beta diversity of the GM.¹⁴

Firmicutes:Bacteroidetes ratio

Firmicutes and Bacteroidetes are the 2 predominant bacterial phyla in the human gut, collectively accounting for approx. 90% of the GM.⁵ An early hypothesis suggested that obesity and metabolic disorders were associated with an increased Firmicutes:Bacteroidetes ratio. In a systematic review by Slouha et al., a trend toward a higher Firmicutes:Bacteroidetes ratio in individuals with elevated BMI was reported; however, this association was not statistically significant (Spearman's $\rho = 0.042$, $p = 0.579$).¹

Given that dietary fiber is predominantly metabolized by Bacteroidetes species, which produce beneficial SCFAs, this hypothesis appears biologically plausible.⁴ Moreover, although lipopolysaccharides (LPS) derived from Gram-negative bacteria were previously thought to promote inflammation, recent evidence suggests that Bacteroidetes may produce less pro-inflammatory or even anti-inflammatory LPS subtypes.¹⁵ Some studies have suggested that certain Firmicutes taxa may exhibit greater efficiency in extracting energy from dietary substrates, potentially contributing to obesity. Additionally, reductions in the abundance of Firmicutes or Bacteroidetes may promote the overgrowth of pro-inflammatory Proteobacteria, thereby exacerbating inflammatory processes.¹⁶ However, findings have been inconsistent, and the Firmicutes:Bacteroidetes ratio is now widely regarded as an oversimplified marker of gut dysbiosis.⁵

Taxonomic changes

As phylum-level analyses are increasingly regarded as oversimplified, research has shifted toward the investigation of specific taxonomic alterations. A systematic review encompassing 20 cohorts confirmed statistically significant changes in GM composition among individuals with T2DM, characterized by reduced abundances of beneficial taxa, including *Akkermansia*, *Faecalibacterium*, *Bifidobacterium*, *Bacteroides*, and certain *Clostridiales*. Conversely, T2DM was associated with significant enrichment of pro-inflammatory and opportunistic bacteria, particularly *Escherichia–Shigella* (often analyzed as a combined group due to 16S rRNA gene sequence similarity), *Fusobacterium*, *Enterococcus*, *Ruminococcus*, and *Dorea*. Interestingly, some studies also reported increased abundance of *Lactobacillus* species. Increased abundance of Gram-negative bacteria may contribute to enhanced endotoxin release, whereas reduced levels of *Faecalibacterium* may decrease butyrate production, a key anti-inflammatory metabolite and primary energy source for colonocytes.^{5,9,17}

Moreover, studies indicate that, despite geographic and ethnic variability, common microbial patterns consistently emerge in T2DM, including reduced abundance of *Faecalibacterium* and increased abundance of *Escherichia*.⁹ A metagenomic meta-analysis further identified microbial

gene signatures associated with butyrate synthesis and LPS production, which distinguished individuals with T2DM from healthy controls.^{2,9}

Bacteroides

The human GM can be broadly categorized into *Bacteroides*- and *Prevotella*-dominant enterotypes, with *Bacteroides*-dominant enterotypes being associated with high-fat, high-protein dietary patterns and reduced microbial diversity.¹⁸ Although *Bacteroides*-dominant enterotypes broadly correlate with dietary patterns and metabolic health, the effects at the genus and species levels vary considerably. The *Bacteroides* enterotype has been reported to be negatively correlated with levels of the tight junction scaffolding protein zonula occludens-1 (ZO-1) ($r = -0.22$ to -0.53 , $p < 0.05$) and SCFAs ($p < 0.05$). Elevated serum ZO-1 levels in individuals with T2DM may indicate impaired gut barrier integrity.¹⁹

Five cross-sectional studies^{20–24} reported negative associations. For example, Munukka et al.²⁰ identified a significant inverse correlation between *Bacteroides* abundance and BMI ($r = -0.28$, $p = 0.011$). In contrast, the 3 interventional studies^{25–27} reported positive associations, potentially reflecting the antibiotic-like effects of metformin on the GM ($p < 0.05$). Additionally, *Bacteroides* abundance has been shown to be inversely correlated with BCAA levels, while therapeutic interventions may improve BCAA metabolism and reduce obesity. Animal studies have demonstrated that administration of *Bacteroides* significantly enhances valine oxidation in brown adipose tissue ($p < 0.05$), thereby promoting more efficient BCAA catabolism.^{4,28} Notably, He et al. reported that 21 of 23 *Bacteroides* operational taxonomic units were negatively associated with T2DM.²⁹

At the species level, *Bacteroides intestinalis* and *Bacteroides vulgatus* have been reported to be reduced in T2DM, whereas *Bacteroides stercoris* increases following surgery in patients with diabetes remission.^{24,25,30,31} Animal studies have shown that *Bacteroides acidifaciens* and *Bacteroides uniformis* improve glucose intolerance ($p < 0.05$),³² whereas *Bacteroides fragilis* induces interleukin-10 (IL-10), thereby protecting against IR ($p < 0.05$). This effect appears to be mediated through the toll-like receptor 2 (TLR2) signaling pathway, as no significant increase in IL-10 was observed in mice lacking this receptor.³³ *Bacteroides fragilis* has also been proposed as a key mediator of metformin's metabolic effects.²⁷ Furthermore, *Bacteroides thetaiotaomicron* enhances regulatory T-cell (Treg) gene expression, thereby reducing inflammation,³³ while *B. vulgatus* and *Bacteroides dorei* improve gut barrier integrity, lowering LPS production and endotoxemia ($p < 0.05$).³⁴ Additionally, *B. acidifaciens* enhances fatty acid oxidation through the TGR5–PPAR- α signaling pathway, thereby alleviating obesity and T2DM.³⁵ These findings underscore the importance of distinguishing the roles of *Bacteroides* across

different taxonomic levels to better understand their metabolic and immunological effects.

Faecalibacterium

Faecalibacterium, which constitutes more than 5% of the total intestinal bacterial population,³⁶ is a SCFA-producing genus that appears to exert protective anti-inflammatory effects and is frequently reported to be reduced in individuals with T2DM.^{5,37} *Faecalibacterium prausnitzii* has also shown potential in reducing IR by inducing glucagon-like peptide-1 (GLP-1) secretion through the free fatty acid receptor 2 (FFAR²).⁴ Additionally, *F. prausnitzii* has been shown to reduce intestinal inflammation by inhibiting nuclear factor kappa B (NF- κ B) activation, suppressing the production of pro-inflammatory cytokines, and enhancing secretion of the anti-inflammatory cytokine IL-10. A recent study further demonstrated that butyrate produced by *F. prausnitzii* exerts anti-inflammatory effects by increasing the expression of *Dact3*, a gene associated with the Wnt/c-Jun N-terminal kinase (JNK) signaling pathway.³⁸

Akkermansia muciniphila

Akkermansia muciniphila also produces SCFAs and influences metabolic pathways such as AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor alpha (PPAR α), thereby regulating metabolism, lipid storage, and inflammation. It promotes gut barrier integrity through interactions with dendritic cells, activation of the ALPK¹/TIFA signaling pathway, and modulation of mucus layer properties. Consequently, *A. muciniphila* is considered a promising next-generation probiotic.^{2,5,39}

Liu et al. conducted a meta-analysis of 15 animal studies investigating the effects of *A. muciniphila* administration as a probiotic. Supplementation was associated with an average reduction in body weight of 10.4%; however, no clear dose–response relationship was observed, and the effects were more pronounced during the early stages of obesity (6–12 weeks). Administration of *A. muciniphila* also reduced fasting blood glucose levels by 21.2% and improved glucose tolerance by 22.1% ($p < 0.001$), with a pooled effect size of 0.43 (95% CI: 0.18–0.63). Moreover, circulating insulin levels increased by 26.9% ($p < 0.001$).⁴⁰ Zhang et al. identified a significant co-occurrence relationship between *Akkermansia* and *F. prausnitzii* ($\rho = 0.36$, $p < 0.05$). This finding suggests potential synergistic benefits of combining these microorganisms as next-generation probiotics.⁴¹

Bifidobacterium

Bifidobacterium is a genus of fiber-fermenting bacteria.^{9,42,43} The relationship between *Bifidobacterium* abundance and T2DM appears to be complex. Several studies have reported reduced abundance of *Bifidobacterium*

species in individuals with metabolic disorders, along with significant associations with greater bacterial richness and reduced intestinal permeability.² However, some studies have linked higher *Bifidobacterium* abundance to hyperlipidemia. Moreover, in individuals with T2DM, correlations have been observed between *Bifidobacterium* abundance, melatonin-related metabolites, and inflammatory markers. Specifically, *Bifidobacterium* abundance was negatively correlated with serum melatonin levels ($p < 0.01$) and positively correlated with inflammatory markers, including LPS and IL-10 (false discovery rate (FDR) < 0.05).¹

Escherichia–Shigella

Bednarska and Håberg reported a positive association between *Escherichia–Shigella* abundance and T2DM across multiple cohort studies, likely mediated by LPS and endotoxin-induced release of pro-inflammatory cytokines through nucleotide-binding oligomerization domain (NOD) and TLR signaling pathways.⁹

A meta-analysis of 32 studies also reported increased abundance of *Escherichia–Shigella* in individuals with obesity ($p < 0.05$).⁸ Additionally, *Escherichia* abundance has been associated with animal-based dietary patterns, whereas fiber-rich, plant-based dietary interventions may reduce its abundance.⁴⁴

Lactobacillus

Lactobacillus and *Bifidobacterium* are common probiotic genera known to contribute to SCFA production, support intestinal barrier integrity, and reduce oxidative stress and inflammation in T2DM animal models, likely through modulation of inflammatory pathways.⁴⁵ However, available evidence remains mixed, with some studies linking *Lactobacillus* abundance to higher fasting blood glucose and glycated hemoglobin (HbA1c) levels in individuals with T2DM. Other authors^{5,46,47} have suggested that *Lactobacillus* may contribute to T2DM progression by promoting GM-related inflammation ($p < 0.05$). Consistent with these findings, a Mendelian randomization study by Liu et al.⁴⁸ also reported a potential association between *Lactobacillus* and increased risk of T2DM. This association may reflect species-specific effects, as certain *Lactobacillus* species may preferentially thrive in high-glucose environments. Alternatively, metformin treatment may promote their growth, suggesting that increased *Lactobacillus* abundance could represent a consequence of treatment rather than a contributing factor in T2DM pathogenesis.⁹

Other species associated with T2DM

Dorea, a genus within the *Lachnospiraceae* family, is frequently reported to be elevated in individuals with T2DM and has been associated with a pro-inflammatory

gut environment, potentially expanding as beneficial microbial populations decline. In addition, increased abundances of *Fusobacterium* and *Enterococcus* have been reported in T2DM, along with *Ruminococcus gnavus*, a mucus-degrading species associated with inflammatory processes.^{5,9}

Gut microbiota-derived metabolites in T2DM

Short-chain fatty acids

Short-chain fatty acids, primarily acetate, propionate, and butyrate, are produced mainly through bacterial fermentation of dietary fiber and resistant starch in the colon.² They serve as an energy source for colonic epithelial cells and function as signaling molecules by interacting with G protein-coupled receptors, including FFAR², FFAR³, and G protein-coupled receptor 109A (GPR109A).^{2,49}

Moreover, SCFAs exert beneficial metabolic effects by enhancing gut barrier integrity, stimulating the secretion of GLP-1 and peptide YY (PYY), regulating glucose and lipid metabolism in peripheral tissues, and modulating the host metabolic state, including appetite regulation.^{1,2,17} For example, butyrate improves insulin sensitivity and exerts anti-inflammatory effects by inhibiting NF- κ B activation. Evidence suggests that butyrate-producing bacteria are depleted in individuals with prediabetes and T2DM.^{2,9,50} Candela et al. reported that a fiber-rich diet significantly increased the abundance of SCFA-producing bacteria, including *Faecalibacterium* and *Akkermansia* ($p = 0.01$).²³ Furthermore, SCFAs activate the SIRT¹/AMPK/PGC-1 α signaling pathway, which may represent a key mechanism underlying their anti-aging effects.⁵¹ Aging-associated alterations in the GM are characterized by reduced SCFA production and amyolytic activity, alongside increased proteolytic activity, thereby contributing to intestinal inflammaging in older individuals.^{52,53} Overall, current evidence suggests that SCFAs may exert beneficial effects in T2DM by enhancing insulin secretion and satiety while reducing inflammation.²

One study further suggests that disturbances in SCFA production or absorption may contribute to the development of T2DM by inducing epigenetic modifications, particularly through alterations in DNA methylation.⁵⁴

Lipopolysaccharide

Lipopolysaccharide, a structural component of the outer membrane of most Gram-negative bacteria, serves as a protective barrier and can translocate into the bloodstream, typically through impaired intestinal barrier integrity, thereby triggering an immune response. Numerous studies

have implicated this mechanism in the development of systemic inflammation.⁵⁵

Metabolic endotoxemia is characterized by persistently elevated circulating LPS levels that promote chronic low-grade inflammation and has been implicated in obesity-associated IR and T2DM. In the setting of gut dysbiosis, e.g., induced by high-fat dietary patterns or frequent antibiotic exposure, LPS-producing bacteria may proliferate, while intestinal barrier function becomes compromised, resulting in increased systemic LPS concentrations.^{56,57} This, in turn, may trigger chronic inflammation within pancreatic islets, ultimately contributing to pancreatic β -cell dysfunction and reduced insulin sensitivity.^{2,58,59}

Lipopolysaccharide activates toll-like receptor 4 (TLR4) expressed on immune and other cell types, thereby triggering the NF- κ B and mitogen-activated protein kinase (MAPK) signaling pathways. In conjunction with TNF receptor-associated factor (TRAF) family E³ ligases, the TLR signaling cascade activates multiple transcriptional regulators, including NF- κ B, activator protein 1 (AP-1), and interferon regulatory factors.⁶⁰ Collectively, these factors drive the expression of genes involved in host immune responses. Activation of these pathways promotes the production of pro-inflammatory cytokines, including tumor necrosis factor alpha (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6). These cytokines disrupt insulin signaling in insulin-responsive tissues and may consequently contribute to pancreatic β -cell dysfunction.^{2,60,61} In individuals with T2DM, elevated levels of inflammatory cytokines and markers of endotoxemia are frequently observed, supporting a link between gut-derived LPS and the chronic inflammatory state associated with the disease.^{2,17,61} Lipopolysaccharide may contribute to the pathogenesis of T2DM by perpetuating systemic inflammation. Mouse models have demonstrated that low-dose LPS infusion significantly promotes weight gain and IR ($p < 0.05$), whereas TLR4-knockout mice or administration of TLR4 antagonists significantly reduce LPS-induced production of pro-inflammatory cytokines, such as IL-6 and TNF- α ($p < 0.01$). Additionally, the GM composition observed in T2DM, characterized by enrichment of Gram-negative *Enterobacteriaceae*, including *Escherichia coli*, may favor increased LPS release.^{2,9}

Furthermore, individuals with T2DM frequently exhibit reduced expression of tight junction proteins in the intestinal epithelium, which may reflect dysbiosis-associated alterations in gut barrier integrity and facilitate LPS translocation.¹ Taken together, these changes contribute to metabolic endotoxemia in T2DM, thereby exacerbating IR. Although therapeutic strategies aimed at reducing LPS burden have demonstrated improvements in metabolic outcomes, a recent animal study targeting macrophages showed that suppression of the inflammatory response alone was insufficient to improve insulin sensitivity ($p > 0.05$).⁶²

Bile acids

Bile acids, the principal components of bile, are essential for the digestion and absorption of lipids, including the absorption of fat-soluble vitamins.⁶³ In addition to their digestive functions, BAs act as signaling molecules involved in a variety of metabolic processes, particularly those regulating lipid and glucose metabolism.⁶⁴

Bile acids also activate multiple BA receptors, including the farnesoid X receptor (FXR), G protein-coupled BA receptor 1 (GPBAR¹), pregnane X receptor (PXR), and vitamin D receptor (VDR). Through these receptors, BAs regulate signaling pathways within a complex metabolic network controlling lipid, glucose, steroid, xenobiotic, and energy metabolism.⁶⁵

In recent years, increasing attention has been directed toward the bidirectional relationship between BAs and the GM. Collins et al. emphasized the importance of BA–GM interactions not only in metabolic disorders, including T2DM, but also in neoplastic diseases and infectious conditions. Probiotic supplementation and antibiotic exposure may also substantially influence this interaction.⁶⁶

Bile acids are frequently highlighted in the literature as key regulators in metabolic disorders, including T2DM, obesity, and prediabetic states. Bishay et al. emphasized the role of BA accumulation in the development of IR.⁶⁷

Recent research from the University Medical Center Schleswig-Holstein (Kiel, Germany) further underscored the interaction between BA metabolism and the GM in metabolic disorders, including T2DM and prediabetes. In a study involving 492 individuals, impaired glycemic control was associated with elevated circulating BA levels, a higher proportion of secondary BAs, and an increased glycine-to-aurine conjugation ratio, particularly for cholic acid, chenodeoxycholic acid, and deoxycholic acid ($p < 0.001$, Kruskal–Wallis test).⁶⁸

Alterations in GM composition, particularly in beta diversity, were significantly associated with changes in the BA pool ($p = 0.0004$), especially with secondary BAs such as deoxycholic acid ($p = 0.001$), independently of T2DM or obesity. Metabolic pathway differences associated with T2DM were primarily observed in bacterial taxa involved in cell membrane synthesis and polyamine production. Collectively, these findings suggest that targeting BA metabolism may represent a promising therapeutic strategy for prediabetes and T2DM.⁶⁸

Dysbiosis in T2DM, particularly in cases characterized by depletion of *Clostridia* and *Bifidobacterium* species possessing bile salt hydrolase activity, may result in reduced BA deconjugation, thereby affecting farnesoid X receptor (FXR) signaling. Interestingly, interventions aimed at modulating BA pools have demonstrated metabolic effects. For example, a recent study involving a non-absorbed BA sequestrant in prediabetic animal models delayed diabetes onset by altering gut BA composition and improving glycemic control.²⁷

Although not all studies have demonstrated a significant association between GM-mediated BA alterations and T2DM development, the available evidence consistently supports an interaction between these mechanisms in metabolic regulation. Nonetheless, targeting BA receptors, such as FXR and Takeda G protein-coupled receptor 5 (TGR5), through microbiota-based or pharmacological interventions remains an active area of research in metabolic disease.

Trimethylamine N-oxide

Trimethylamine N-oxide is predominantly generated from dietary substrates, including phosphatidylcholine, choline, carnitine, betaine, dimethylglycine, and ergothioneine, through GM-mediated metabolism in the colon. Its circulating levels are influenced by multiple factors, including age, sex, dietary patterns, GM composition, renal function, and hepatic flavin-containing monooxygenase activity.⁶⁹

Trimethylamine (TMA), produced by gut bacterial metabolism of choline and L-carnitine, is absorbed into the bloodstream and subsequently rapidly oxidized to TMAO by hepatic flavin-containing monooxygenase 3 (FMO3).⁷⁰

In recent years, increasing attention has been directed toward the association between the GM, its metabolites, including TMAO, and a broad range of diseases, particularly metabolic and cardiovascular disorders.⁷¹ Elevated TMAO levels may result from dietary factors, alterations in GM composition, dysbiosis, or impaired gut barrier function. Advances in sequencing technologies have substantially expanded our understanding of the GM and its role in health and disease. Diet plays a crucial role in shaping GM composition, thereby influencing the production of metabolites such as SCFAs and TMAO. While TMAO has been associated with an increased risk of cardiovascular disease (CVD) and mortality ($p < 0.001$), SCFAs are generally considered protective in metabolic health, and these metabolites should therefore not be grouped together in terms of risk interpretation. Bacterial taxa implicated in TMA production include members of *Clostridia*, *Proteus*, *Shigella*, and *Aerobacter*.⁷⁰

In the gastrointestinal tract, certain bacterial taxa, such as *Acinetobacter*, can metabolize lecithin and carnitine to produce TMA, which is subsequently oxidized to TMAO in the liver. Analyses of fecal samples have shown that individuals with higher abundance of *Prevotella* species, often associated with high-fat dietary patterns, exhibit higher TMAO levels compared with those with *Bacteroides*-dominant microbiota.⁷²

A growing body of evidence has demonstrated associations between TMAO, a GM-derived metabolite, and various lifestyle-related diseases, including IR, T2DM, and obesity.^{73–75} Kalagi et al. also emphasized the significant association between TMAO and T2DM.⁷⁶

A study comparing individuals with T2DM and healthy controls reported significantly higher median plasma

TMAO levels in the T2DM group ($p < 0.001$). This association remained significant after adjustment for age, BMI, and educational level. Even after accounting for physical activity and dietary factors, elevated TMAO levels were associated with a higher risk of T2DM. These findings suggest that increased circulating TMAO concentrations may be associated with T2DM.⁷⁷

Tryptophan metabolites (indoles)

Indole-3-propionic acid (IPA) is one of the most abundant tryptophan-derived metabolites in the human gastrointestinal tract.⁷⁸ Evidence suggests that IPA may mitigate the progression of metabolic disorders, including non-alcoholic fatty liver disease, T2DM, obesity, and CVD ($p < 0.05$ or $p < 0.01$, depending on the experimental model).⁷⁹ Its proposed protective effects include modulation of inflammatory responses, reduction of oxidative stress, limitation of DNA damage, regulation of lipid metabolism, and support of immune function.^{80,81}

A cohort study by Qi et al. found that tryptophan, 4 kynurenine pathway metabolites (kynurenine, kynurenic acid, xanthurenic acid, and quinolinic acid), and indolelactate were positively associated with T2DM risk ($p < 0.05$), whereas IPA was inversely associated ($p < 0.001$). The study also identified genetic variants, dietary factors, and gut microbial interactions linked to these metabolites. Notably, dietary fiber intake showed the strongest association with tryptophan-derived metabolites, particularly IPA, which was associated with *Firmicutes* taxa involved in fiber metabolism.⁷⁷

Moreover, a murine study demonstrated that GM-derived indoles influence intestinal barrier function through pregnane X receptor (PXR) signaling. Indole-3-propionic acid acts as a ligand for PXR, reducing TNF- α expression in enterocytes and enhancing the expression of intercellular junction proteins. PXR-deficient mice exhibited increased intestinal permeability and activation of the TLR signaling pathway; these effects were reversed by additional TLR4 silencing. Collectively, these findings suggest that GM regulate intestinal mucosal integrity through interactions involving the PXR and TLR4 signaling pathways.⁸²

Several additional studies have also highlighted the role of indoles as signaling molecules that modulate GLP-1 secretion by enteroendocrine L cells in the colon,⁸³ thereby influencing appetite regulation and potentially improving insulin sensitivity.⁸⁴

Dysbiosis and gut permeability

Dysbiosis is defined as a disruption of the balance between symbiotic and pathobiont microbial populations, characterized by an increase in pathogenic microorganisms.^{22,23} Alterations in the GM can impair intestinal barrier integrity and increase gut permeability, thereby

facilitating the translocation of metabolites and pathogens from the intestine into the systemic circulation and peripheral tissues. Dysbiosis has been associated with increased adiposity, IR, enhanced energy harvest, elevated production of pro-inflammatory cytokines, and increased LPS translocation leading to metabolic endotoxemia. It may contribute to the pathogenesis of conditions such as obesity and T2DM, both of which are associated with systemic inflammation.^{42,85} Increased intestinal permeability is considered an early event in the pathogenesis of T2DM and may be influenced by GM alterations. A controlled study found that individuals with T2DM matched for body weight, age, and BMI still exhibited increased intestinal permeability and higher bacterial endotoxin levels compared with individuals without diabetes.^{5,86}

Alterations in microbial diversity and shifts in the relative abundances of specific bacterial taxa have been shown to influence intestinal permeability. For instance, *A. muciniphila* has been shown to restore the intestinal mucosal layer.⁹ Furthermore, studies suggest that *Lactobacillus* species may enhance barrier integrity by regulating the expression of tight junction (TJ) proteins.⁸⁶ *Bifidobacterium* administration has been shown to markedly decrease intestinal permeability by increasing TJ protein expression and reducing levels of inflammatory cytokines, including IL-6 and TNF- α .^{2,9}

Additionally, research indicates that SCFAs and BAs can activate multiple key receptors expressed by enteroendocrine cells, thereby increasing the secretion of GLP-1, glucagon-like peptide-2 (GLP-2), and PYY. This, in turn, may contribute to reduced blood glucose levels and improved intestinal barrier integrity.⁸⁷ For instance, *Roseburia* has been shown to improve glucose homeostasis and intestinal permeability through SCFA production.² Moreover, a reduced abundance of the *Haemophilus* genus in the intestine may promote SCFA production, potentially contributing to improved intestinal barrier function.^{48,87}

BMI association with gut microbiota in T2DM

Obesity is a chronic, complex disease characterized by excessive adipose tissue accumulation that may impair health and reduce quality of life. The World Health Organization (WHO) defines obesity in adults as a BMI of 30 kg/m² or higher. Obesity is a major risk factor for T2DM, and a substantial proportion of individuals with this condition are overweight or obese.⁸⁵ Both conditions are associated with GM disturbances and chronic low-grade inflammation. Disentangling the effects of elevated BMI and hyperglycemia on GM composition remains challenging, as these factors frequently coexist.⁴² Studies suggest that obesity itself induces specific alterations in the GM that may contribute to IR and metabolic dysfunction independently of diabetes.^{3,86}

High-carbohydrate dietary patterns, commonly observed in plant-based diets, tend to increase *Prevotella* abundance, which has been associated with more favorable glucose metabolism. Moreover, individuals with elevated BMI and diets high in fat and protein have been reported to exhibit an increased Firmicutes:Bacteroidetes ratio.⁴² In the context of T2DM, Carrizales-Sánchez et al. have observed a direct positive correlation between *Prevotella* abundance and BMI.⁸⁷ Furthermore, Slouha et al. found that *A. muciniphila* levels were higher in individuals with T2DM and elevated BMI, and this was significantly associated with impaired lipid metabolism.¹ This observation appears to contradict the established view that *Akkermansia* is generally beneficial, as its abundance is typically higher in lean individuals and lower in those with obesity.³ Elevated levels of *Blautia* and *Serratia*, along with reduced *Akkermansia*, have been observed in individuals with prediabetes and T2DM compared with normoglycemic controls, even when dietary intake and caloric consumption were similar across groups.⁴⁰ These findings suggest that, in certain contexts, *Akkermansia* may increase in abundance in response to host metabolic disturbances, indicating a complex and context-dependent role. It is noteworthy that not all GM alterations associated with T2DM simply reflect obesity. While obesity-associated dysbiosis predisposes individuals to IR and T2DM, 3 certain microbial features appear to be associated with glycemic control independently of BMI. For instance, both lean and obese individuals with diabetes exhibit overgrowth of *Escherichia-Shigella* and reduced *Faecalibacterium* abundance.^{5,9} These observations indicate that GM alterations in T2DM extend beyond obesity-related effects and warrant further investigation.

Dietary patterns and gut microbiota in T2DM

The Western diet is characterized by high intake of animal fats, low dietary fiber consumption, and an increased risk of gut dysbiosis, obesity, and T2DM.¹ Following diets rich in animal fats and red meat, the GM ferments choline, carnitine, betaine, and lecithin to produce TMA, which is subsequently metabolized in the liver to TMAO. This metabolite has been shown to impair glucose tolerance and increase homeostatic model assessment of IR (HOMA-IR) and fasting insulin levels in mice fed a high-fat diet, thereby promoting adipose tissue inflammation and disrupting insulin signaling.^{5,9,17} Moreover, high-fat, low-fiber dietary patterns have been associated with reduced abundance of Bacteroidetes and increased abundance of Firmicutes and Proteobacteria.² Specifically, such diets may increase the abundance of pro-inflammatory bacteria, such as *Alisipies* and certain *Bacteroides* species, while reducing beneficial taxa, including *Faecalibacterium*, potentially through mechanisms involving elevated LPS levels.^{1,3,23}

In contrast, diets rich in complex carbohydrates and dietary fiber support a more diverse and resilient GM, increasing the relative abundance of SCFA-producing bacteria, such as *Bifidobacterium*, *F. prausnitzii*, and *Akkermansia*.⁴ Chong et al. observed that a high-fiber diet was associated with metabolic improvements. Dietary fiber intake has been associated with favorable effects on GM composition and improved metabolic homeostasis in individuals with T2DM.⁵ Furthermore, decreased fiber intake has been linked to a higher risk of T2DM. Contemporary dietary recommendations for diabetes management emphasize diets rich in plant-based foods, including whole grains, vegetables, whole fruits, legumes, nuts, and seeds. High-fiber dietary interventions have been associated with significant improvements in HbA1c and body weight reduction, potentially through the promotion of SCFA-producing bacteria.⁹

High-fiber dietary interventions in individuals with T2DM have also been associated with increased abundance of SCFA-producing bacteria. Enhanced SCFA production may promote increased GLP-1 secretion and reduced inflammatory activity, thereby contributing to improved glycemic control.^{4,5,9}

Mediterranean diet

The Mediterranean diet is widely recognized as one of the healthiest dietary patterns and has been associated with a reduced risk of T2DM and better glycemic control.^{6,85} The Mediterranean diet is rich in vegetables, fruits, legumes, whole grains, nuts, and healthy fats from olive oil and avocados, contains low amounts of red meat, and excludes processed foods and sugars. Plant-based foods are rich in polyphenols, flavonoids, and terpenoids, bioactive compounds that have been shown to exert nutrigenomic effects by modulating gene expression via epigenetic mechanisms.⁴² Polyphenols act as antioxidants, combating oxidative stress and chronic inflammation, while also improving insulin sensitivity.^{5,9,17}

Adherence to the Mediterranean diet has been shown to increase the diversity of GM by modulating its composition and promoting a microbiome associated with metabolic health, reduced dysbiosis, and improved intestinal barrier integrity.^{85,87} A study conducted by Özsoy et al. found that individuals following the Mediterranean diet have higher levels of *Bifidobacteria*, *Lactobacilli*, and SCFA-producing bacteria such as *Faecalibacterium* and *Roseburia*, along with lower levels of pro-inflammatory bacteria compared to those following the Western diet.⁸⁵ Moreover, the Mediterranean diet has been associated with an increased *Prevotella/Bacteroides* ratio, potentially promoting gut homeostasis and improving glucose metabolism and insulin secretion.⁸⁷ A study by Bednarska and Håberg found the Mediterranean diet to be effective in reducing fasting blood glucose and HbA1c. Given its beneficial effects on glycemic control, the Mediterranean diet is considered a preferred dietary pattern for T2DM management.⁹

Low-carbohydrate and ketogenic diets

The ketogenic diet, characterized by very low carbohydrate and high fat intake, effectively lowers HbA1c levels. This may be associated with increased abundance of *Akkermansia* and *Parabacteroides*.^{2,4} The low-carbohydrate diet exhibits glycemic improvement largely by reducing exogenous glucose load rather than by promoting a high-SCFA-producing microbiome. Moreover, this diet has been associated with significantly higher relative abundances of *Ruminococcus*, *Roseburia*, and *Eubacterium*.⁴ *Eubacterium* may adapt to generate SCFAs from available substrates even under low-carbohydrate conditions, potentially contributing to a significant reduction in HbA1c.^{1,4} The mechanisms underlying the ketogenic diet may include elevated GLP-1 levels and improved glucose homeostasis, which may be linked to increased growth of SCFA-producing bacteria such as *Ruminococcus* and *Roseburia*.¹ Moreover, the very-low-calorie ketogenic diet (VLCKD) may induce elevated plasma concentrations of acetoacetic acid (ACA) and β -hydroxybutyric acid (β -OHB), as well as activation of white adipose tissue (WAT) lipolysis. Caloric restriction in individuals with T2DM may also reorganize GM composition and promote SCFA production. Ketogenic diets have additionally been associated with reduced levels of inflammatory T helper 17 (Th¹⁷) cells.^{2,4} The ketogenic diet is being explored for its potential role in the management of obesity and other metabolic disorders.

Effects of probiotics on gut microbiota and glycemic control

Probiotics are living microorganisms that, when consumed in appropriate amounts, exert beneficial effects on the host's health, such as reducing circulating LPS levels, fasting blood glucose, IR, and HbA1c levels. The underlying mechanisms of these beneficial effects remain under investigation. Studies suggest that probiotics decrease intestinal permeability and secretion of pro-inflammatory mediators while increasing production of bacteriocins and SCFAs. Short-chain fatty acids facilitate communication between the intestinal lumen and the rest of the body, thereby regulating energy metabolism and adipose tissue expansion. Through these mechanisms, butyrate-producing taxa may represent a promising therapeutic strategy for managing T2DM.^{88,89}

A 2023 meta-analysis of 22 randomized controlled trials (RCTs) concluded that probiotic supplementation was associated with modest reductions in HbA1c, fasting glucose, and HOMA-IR in individuals with T2DM. It highlighted certain strains, such as *L. rhamnosus* GG and *B. animalis*, as potentially useful adjuncts in diabetes management.⁹⁰

Moreover, various meta-analyses support these findings.^{91,92} However, studies conducted by Paquette et al. failed to establish a consistent pattern of glycemic

improvement, as none of the analyzed controlled trials demonstrated a uniform reduction across all major glycaemic parameters with probiotic use. This lack of consistency in glycaemic outcomes following probiotic intervention may be attributed to differences in probiotic composition, duration of supplementation, and dosage.⁹³

Probiotic strains

Multiple RCTs have demonstrated that specific probiotic strains may improve glycaemic control and enhance insulin sensitivity in individuals with T2DM; however, their composition, optimal dosage, and interactions remain under investigation.¹⁴

Most of the available evidence has been derived from mouse models, with *L. plantarum* and *L. rhamnosus* being the most frequently studied strains. Beneficial effects of *L. reuteri*, *L. casei*, and *L. paracasei* have also been frequently reported.^{94–96} Moreover, when combined with *Bifidobacterium* species, these strains may exhibit synergistic effects, potentially contributing to diabetes prevention.⁹⁷

Lactobacillus and *Bifidobacterium* are among the most extensively studied probiotic genera and have been shown to influence T2DM progression through multiple mechanisms, including increasing GLP-1 secretion, decreasing cholesterol absorption, regulating pancreatic β -cell activity, and reducing hepatic gluconeogenesis.^{95,96,98} Campaniello et al. observed that combining *Lactobacillus*, *Bifidobacterium*, and *Streptococcus thermophilus* strains improves lipid profiles and lowers fasting glucose levels in humans.⁹⁴ Likewise, Tegegne et al.⁹⁵ reported in their meta-analysis that combinations of probiotic strains may inhibit weight gain. Furthermore, Li et al.⁹⁹ suggested that the synergistic effects of *Lactobacillus* and *Bifidobacterium* contribute to reductions in HbA1c and markers of oxidative stress. Consistent with these findings, Shen et al.¹⁰⁰ also reported beneficial effects of probiotic combinations on body mass.

Furthermore, *A. muciniphila* has been shown to contribute to restoration of the intestinal mucosal layer and is believed to reduce intestinal permeability, thereby exerting anti-inflammatory effects as a probiotic species and potentially reducing the risk of T2DM.^{9,40,101} Other bacteria with a potentially beneficial impact on glucose metabolism include *F. prausnitzii* and *S. thermophilus*.¹⁰² Analyzed data regarding the role of selected probiotic strains, their influence on T2DM progression parameters, and the study samples used are summarized in Table 1.^{9,40,90,94–99,102}

Additionally, genetic engineering methods such as CRISPR/Cas⁹ are increasingly being used to modify probiotic genomes and enhance the functionality of specific strains. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) systems are being used to edit species such as *Clostridium butyricum* and *Bacteroides* strains, contributing to a better understanding of beneficial metabolic pathways influencing gut permeability and metabolite production. Although inserting DNA into

certain bacterial species remains challenging, CRISPR is considered a promising tool for next-generation microbiome therapies.¹⁰³

Probiotics' mechanisms of influencing T2DM

Probiotics influence the progression of diabetes through various mechanisms, such as enhancing intestinal barrier integrity, alleviating oxidative stress, modulating immune responses, and promoting the production of SCFAs.¹⁰⁴

The primary pathway involves the interaction of SCFAs with G protein-coupled receptors (GPCRs), particularly GPR⁴¹ and GPR.⁴³ Activation of GPR⁴³ enhances insulin sensitivity and reduces inflammation by promoting glucose uptake and stimulating GLP-1 secretion.^{105,106} Moreover, SCFAs act through peroxisome proliferator-activated receptors (PPARs), which modulate insulin signaling in the liver, adipose tissue, and skeletal muscle, potentially improving insulin sensitivity. In the liver, PPAR α promotes fatty acid oxidation and reduces triglyceride accumulation, which may impair insulin signaling. Moreover, PPAR β/δ increases fatty acid utilization in skeletal muscle, thereby supporting improved glucose uptake.^{107,108} Additionally, probiotics have been associated with increased secretion of PPY which may reduce IR and improve β -cell function.^{109,110}

Moreover, *Lactobacillus* and *Bifidobacterium* promote intestinal colonization by *Muribaculaceae*, a mucin glycan- and dietary fiber-metabolizing bacterial family that produces SCFAs beneficial to gut health, whose abundance decreases in obesity and T2DM but may be supported by plant-based diets and probiotic supplementation.¹¹¹ Although the exact mechanisms remain unclear, probiotics may reduce leptin levels, thereby decreasing food intake and promoting fat mass reduction. Consequently, this may improve insulin sensitivity in peripheral tissues.¹¹²

Furthermore, probiotics may influence the expression of glucose transporter type 4 (GLUT-4) and contribute to reactive oxygen species (ROS) neutralization, enzyme activity inhibition, and metal ion chelation, thereby exerting antioxidant effects.¹¹³ In RCTs, probiotics have been shown to reduce serum interleukin-18 (IL-18) levels and improve kidney function in individuals with T2DM, potentially by limiting activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, which is activated by this interleukin.¹¹⁴

Prebiotics

Prebiotics are non-viable dietary substrates that are selectively utilized by host microorganisms to confer health benefits. Early research focused mainly on carbohydrate-based compounds such as inulin, fructo-oligosaccharides (FOS), and galacto-oligosaccharides (GOS), which are not digested in the small intestine but are instead fermented by gut bacteria to produce SCFAs.^{89,93}

Table 1. Effects of probiotics in type 2 diabetes mellitus (studies in humans and mice)

Reference number	Authors	Sample	Probiotic strains used	Observed effects
9	Bednarska and Häberg	mice	<i>Akkermansia muciniphila</i>	↓ IR, ROS ↑ glucose tolerance
40	Liu et al.	mice	<i>Akkermansia muciniphila</i>	↓ FG ↑ glucose tolerance, insulin
90	Ayesha et al.	humans	<i>Lactobacillus paracasei</i> <i>Lactobacillus rhamnosus</i> <i>Bifidobacterium animalis</i>	↓ FG, IR, HbA1c, LPS improved lipid profile
94	Campaniello et al.	humans	<i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus bulgaricus</i> , <i>Bifidobacterium breve</i> , <i>Bifidobacterium longum</i> , <i>Streptococcus thermophilus</i>	↓ FG ↑ HDL
94	Campaniello et al.	mice	<i>Lactobacillus paracasei</i> <i>Lactobacillus rhamnosus</i> <i>Lactobacillus kefiranofaciens</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus helveticus</i> , <i>Lactobacillus lactis</i> , <i>Issatchenkia orientalis</i>	↓ FG, LPS, TNF-α, IL-6, hsCRP ↓ FG, IR, LPS ↓ FG, HbA1c, improved lipid profile, glucose tolerance ↑ C-peptide
95	Tegegne et al.	mice	<i>Bifidobacterium lactis</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus gasseri</i>	↓ LPS, IR improved lipid profile
96	Sarmadi et al.	mice	<i>Lactobacillus casei</i> , <i>Lactobacillus plantarum</i> , <i>Lactobacillus rhamnosus</i> , <i>Lactobacillus brevis</i> , <i>Lactobacillus plantarum</i> <i>Lactobacillus plantarum</i> <i>Lactobacillus fermentum</i> <i>Lactobacillus rhamnosus</i>	↓ FG, HbA1c, IR, leptin, TNF-α, IL-6, IL-10 ↑ glucose tolerance ↑ GLP-1, C-peptide
96	Sarmadi et al.	humans	<i>Lactobacillus acidophilus</i> <i>Lactobacillus casei</i> <i>Bifidobacterium bifidum</i>	↑ insulin, HDL
97	Jeyaraman et al.	humans	<i>Lactobacillus acidophilus</i> <i>Lactobacillus brevis</i> <i>Lactobacillus casei</i> <i>Lactobacillus salivarius</i> <i>Lactobacillus lactis</i> <i>Lactobacillus lactis</i> <i>Bifidobacterium bifidum</i> <i>Bifidobacterium lactis</i>	↓ FG, IR
98	Leser et al.	mice	<i>Bifidobacterium adolescentis</i>	↓ FG, IR, ROS
99	Li et al.	humans	<i>Lactobacillus casei</i>	↓ FG, IR, ROS
102	Tiwari et al.	mice	<i>Faecalibacterium prausnitzii</i> <i>Streptococcus thermophilus</i>	↓ FG, ROS

FG – fasting glucose; IR – insulin resistance; ROS – reactive oxygen species; HbA1c – hemoglobin A1c; HDL – high-density lipoprotein; LPS – lipopolysaccharide; GLP-1 – glucagon-like peptide-1; TNF-α – tumor necrosis factor alpha; IL-6 – interleukin-6; IL-10 – interleukin-10; hsCRP – high-sensitivity C-reactive protein.

Moreover, these metabolites contribute to pathogen inhibition and reinforcement of gut barrier integrity through end products such as acetate and lactate.^{93,103} Prebiotics also promote the proliferation of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, while suppressing the growth of potentially detrimental taxa, such as *E. coli* and *Clostridium*. Hence, prebiotics contribute to a more stable intestinal environment. This modulation of the GM by prebiotics has also been associated with reduced levels of uremic toxins and systemic inflammation, suggesting

broader relevance in chronic kidney disease (CKD) and other metabolic conditions.¹¹⁴

These effects have been linked to improvements in glyce-mic parameters in T2DM, including reductions in fasting glucose (FG), HbA1c, and IR. A study by Garcia-Gutierrez et al. highlighted that beneficial effects were most pronounced, particularly with longer inulin interventions lasting 8 weeks or more.⁸⁹ Furthermore, inulin may exert additional metabolic benefits by modulating GLP-1 secretion and reducing inflammatory markers. Li et al. observed

that combining probiotics and prebiotics into synbiotics may yield synergistic effects, more effectively lowering FG and inflammatory markers compared with either component alone.⁹⁹

Moreover, higher-dose prebiotic supplementation (≥ 10 g/day) has demonstrated a more beneficial impact on lipid profiles, including total cholesterol, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), high-density lipoprotein (HDL), and triglycerides. This effect is likely mediated through restoration of microbial diversity, which is progressively diminished by Westernized, ultra-processed dietary patterns worldwide.¹⁰⁷

Limitations of the study

This review is limited by the fact that most available data are derived from geographically restricted populations. Additionally, phylum-level analyses often overlook strain-level differences that are crucial to host–microbe interactions. Mechanistic understanding is further constrained by the limited availability of biochemical and molecular tools for direct investigation of metabolite–receptor interactions. The limited number of longitudinal studies and inconsistent findings across cohorts highlight the need for standardized, large-scale investigations.

Conclusions

Type 2 diabetes mellitus and the GM have an intertwined relationship, with each influencing the imbalance of the other. Gut dysbiosis impairs glucose homeostasis and promotes systemic inflammation through increased intestinal permeability, decreased SCFA production, dysregulated BA signaling, and elevated TMAO and LPS levels, while hyperglycemia and obesity further remodel microbial composition. Studies have observed an overall reduction in microbial diversity and enrichment of pro-inflammatory genera, most notably *Escherichia–Shigella*. Higher BMI has been associated with an increased Firmicutes:Bacteroidetes ratio and elevated *A. muciniphila* abundance in individuals with T2DM, which has been linked to impaired lipid metabolism. Moreover, decreased *F. prausnitzii* abundance has been associated with IR, potentially through reduced GLP-1 induction. These microbial alterations underscore a potential association between T2DM and the GM. Probiotic and prebiotic interventions have demonstrated improvements in glycemic control and gut barrier integrity in T2DM. Genetic engineering approaches, such as CRISPR/Cas9-mediated probiotic genome modification, may offer potential for more targeted microbiome-based therapies. The relationship between the GM and T2DM remains incompletely understood and requires further focused investigation. Recent evidence suggests that certain bacterial taxa may influence insulin secretion and IR, thereby contributing to T2DM

pathogenesis. Future research should focus on functional analyses of gut microbes, as well as the effects of probiotics and lifestyle modifications, to improve metabolic outcomes in T2DM.

Use of AI and AI-assisted technologies

Not applicable.

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Global trends and evidence on dyslipidemia in ischemic stroke: An integrative scientometric and meta-analytic review

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Conflict of interest

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Abstract

Dyslipidemia has been identified as an important modifiable risk factor for ischemic stroke (IS); however, inconsistent findings have limited its translational application. This study aimed to integrate scientometric and meta-analytic approaches to provide a comprehensive understanding of lipid abnormalities in IS. A scientometric analysis of 1,962 publications published between 2005 and 2024 revealed evolving research trends, ranging from traditional studies of lipid metabolism to investigations of molecular mechanisms involving high-density lipoprotein (HDL) dysfunction, lipidomics, and precision prevention. A meta-analysis of 13 eligible studies demonstrated that patients with IS had significantly higher levels of total cholesterol (TC; mean difference (MD) = 0.94 mmol/L, 95% confidence interval (95% CI): 0.36–1.52), triglycerides (TG; MD = 0.37 mmol/L, 95% CI: 0.19–0.55), and low-density lipoprotein cholesterol (LDL-C; MD = 0.66 mmol/L, 95% CI: 0.33–0.99), as well as lower levels of high-density lipoprotein cholesterol (HDL-C; MD = –0.30 mmol/L, 95% CI: –0.41 to –0.18), with all comparisons reaching statistical significance ($p < 0.001$). These findings indicate a strong association between dyslipidemia and the risk of IS. The integration of bibliometric and quantitative evidence underscores the importance of comprehensive lipid screening and management in stroke prevention and provides a dual-framework model linking academic research trends with clinical decision-making.

Key words: dyslipidemia, ischemic stroke, evidence synthesis, precision prevention, lipid markers

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Highlights

- This integrative review confirms a strong association between dyslipidemia and an increased risk of ischemic stroke (IS).
- Patients with IS show significantly higher levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C), alongside lower levels of high-density lipoprotein cholesterol (HDL-C).
- Scientometric analysis reveals a research shift toward HDL dysfunction, lipidomics, and precision prevention in stroke research.
- The findings support comprehensive lipid screening and targeted lipid management as key strategies for IS prevention.

Introduction

Ischemic stroke (IS) is a leading global cause of death and long-term disability, accounting for approx. 85% of all stroke cases worldwide.^{1,2} According to the World Health Organization (WHO), stroke is the 2nd leading cause of death globally, with approx. 6 million deaths annually attributed to stroke, of which about 85% are due to IS.³ With the ongoing aging of the global population, the incidence of stroke is expected to increase further.⁴ Dyslipidemia, particularly characterized by elevated total cholesterol (TC), elevated triglycerides (TG), low levels of high-density lipoprotein cholesterol (HDL-C), and elevated low-density lipoprotein cholesterol (LDL-C), has been identified as a modifiable and measurable risk factor for both cardiovascular and cerebrovascular diseases.^{5–8} Despite extensive research, inconsistencies remain regarding the role of lipid abnormalities in the development of IS. These inconsistencies may be attributable to differences in study design, sample size, ethnic background, and diagnostic criteria. Importantly, there remains a lack of integration between macro-level research trends and quantitative clinical evidence.^{9,10}

Scientometric analysis has emerged as a powerful tool for mapping the evolution of research and identifying emerging research hotspots,¹¹ while meta-analysis provides a statistical synthesis of findings across studies.^{12,13} However, few studies have combined these approaches¹⁴ to provide a comprehensive overview of the relationship between dyslipidemia and stroke.^{15–17} Although multiple epidemiological studies have reported that elevated LDL-C and TG levels are associated with an increased risk of stroke, the precise magnitude of their contribution and the interactions among lipid subtypes remain unclear. Moreover, recent molecular and lipidomic studies suggest that HDL functionality, rather than its absolute concentration, may play a critical role in vascular protection. These findings highlight the need for an updated synthesis that bridges clinical and molecular perspectives.

Globally, differences in diet, genetics, and access to healthcare have contributed to geographic variations in lipid profiles and stroke patterns. For example, Asian populations tend to exhibit higher TG levels and lower HDL-C levels than Western populations, potentially

modifying the strength of the association between dyslipidemia and stroke. In addition, the widespread use of statins and other lipid-lowering therapies has highlighted the importance of understanding how treatment may modify risk estimates in contemporary prevention strategies.

From the perspective of research evolution, scientometric methods can capture shifts in academic priorities, ranging from atherosclerosis and oxidative stress to precision medicine, lipidomics, and artificial intelligence (AI)-assisted risk modeling. Integrating these bibliometric trends with quantitative meta-analytic evidence provides a dual framework for interpreting both the evolution of scientific knowledge and its clinical relevance.

Therefore, a combined scientometric and meta-analytic approach is needed to comprehensively evaluate the relationship between lipid abnormalities and IS. Such an approach not only identifies the most influential contributors and emerging research topics but also quantifies the overall strength of evidence across populations and study designs.

Objectives

This study aimed to address this gap by integrating scientometric and meta-analytic approaches to: 1) map global research trends in lipid-related stroke research; 2) assess the pooled associations between lipid markers and the risk of IS; and 3) identify implications for precision prevention and clinical decision-making. This dual-framework approach provides a more comprehensive understanding of both developments in the scientific literature and clinically actionable evidence in this field.

Materials and methods

Scientometric data collection and analysis

A scientometric analysis was conducted using the Web of Science Core Collection database. Literature published between January 1, 2005, and October 10, 2024, was retrieved. The search strategy was based on the following query:

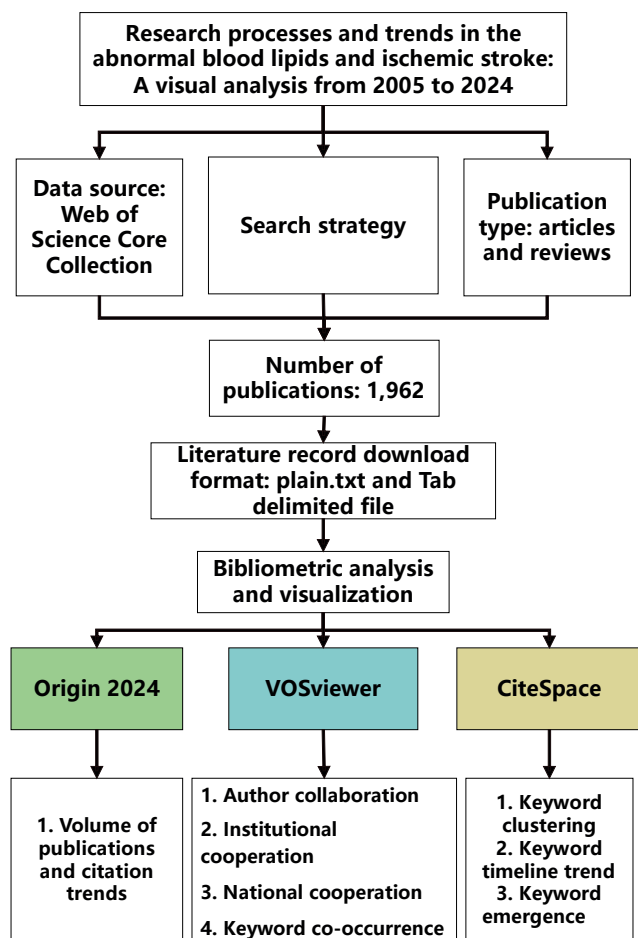


Fig. 1. Workflow of the scientometric and meta-analytic procedures

TS = (“hyperlipidemia” OR “dyslipidemia” OR “high cholesterol” OR “high triglycerides”) AND (“ischemic stroke” OR “cerebral infarction” OR “cryptogenic stroke”). Only original articles and review articles published in English or Chinese were included. Other publication types, such as editorials, letters, and conference abstracts lacking complete data, were excluded. To ensure the inclusion of influential publications, a minimum citation threshold of 10 citations was applied. Records lacking substantive academic content were also excluded. Following the screening process, 1,962 eligible publications were included in the scientometric analysis and visualization. CiteSpace v. 6.1.R6 (Drexel University, Philadelphia, USA; <https://citespace.podia.com>) and VOSviewer v. 1.6.20 (Centre for Science and Technology Studies, Leiden University, the Netherlands; <https://www.vosviewer.com>) were used to construct keyword co-occurrence networks, collaboration maps of authors and institutions, and thematic evolution pathways. These analyses enabled the identification of research hotspots, influential contributors, and emerging trends in the field of dyslipidemia and IS (Fig. 1).

Meta-analysis literature search

A systematic literature search was conducted in four databases: PubMed, Web of Science, Chinese National

Knowledge Infrastructure (CNKI), and Wanfang, from inception to December 2024. The search strategy incorporated both MeSH/Emtree terms and free-text keywords related to dyslipidemia and IS. Boolean operators (AND/OR) were used to combine search terms. An example of the PubMed search strategy was as follows: (“Dyslipidemias”[MeSH] OR dyslipid* OR hyperlipid* OR hypercholesterolemia) AND (“Ischemic Stroke”[MeSH] OR cerebral infarct*) AND (“Risk Factors”[MeSH] OR association OR correlation). Eligible studies included observational studies and randomized controlled trials (RCTs) involving adult participants (≥ 18 years) with IS diagnosed according to internationally recognized criteria, such as the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification. All included studies were required to report quantitative lipid parameters, including TC, TG, LDL-C, and HDL-C together with mean values and standard deviations (SDs). Articles published in English or Chinese were considered eligible. Studies were excluded if they involved hemorrhagic stroke, transient ischemic attack (TIA), or animal models; lacked usable quantitative data (e.g., reported only p-values without means or SDs); or were duplicate publications. Studies with inadequate reporting of potential confounding factors were also excluded. The entire review process was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure the transparency and reproducibility of the search and study selection procedures.

Data extraction and quality assessment

Both reviewers independently extracted the following data from each eligible study: author(s), year of publication, country, sample size, participant age, diagnostic criteria, and reported lipid parameters. Any disagreements were resolved through discussion and consensus between both reviewers. Study quality was assessed using the Cochrane Risk of Bias Tool (<https://methods.cochrane.org/bias>). The assessment included domains such as random sequence generation, allocation concealment, blinding, incomplete outcome data, selective reporting, and other potential sources of bias.

Statistical analyses

Meta-analyses were conducted using RevMan v. 5.4 (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark) and R (R Foundation for Statistical Computing, Vienna, Austria: meta and metafor packages; <https://cran.r-project.org/web/packages/meta/index.html> and <https://cran.r-project.org/web/packages/metafor/index.html>). Mean differences (MDs) and 95% confidence intervals (95% CIs) were calculated for lipid levels between IS patients and controls using random effects models due to expected heterogeneity. Heterogeneity was quantified using the I^2 statistic and Tau², with $I^2 > 50\%$ considered substantial.

Sensitivity analyses were performed by sequentially omitting studies. Publication bias was assessed using funnel plots and Egger's regression test ($p < 0.05$ indicating bias).

Results

Scientometric findings

Global publication trends and research focus

From 2005 to 2024, publications on dyslipidemia and IS increased steadily, peaking in 2022 with 172 articles (Fig. 2). Early studies focused on atherosclerosis and cardiovascular risk, whereas more recent research has emphasized precision lipid management, personalized prevention, and secondary stroke risk reduction. China and the USA led global research output, accounting for more than 48% of all publications. Chinese studies prioritized large-scale epidemiological surveillance, while U.S. institutions advanced mechanistic and translational research. Representative journals included *Stroke*, *Journal of Stroke and Cerebrovascular Diseases*, and *Atherosclerosis*.

Author and institutional networks

Author collaboration mapping revealed tightly connected author clusters centered on Bruce Ovbiagele, Ralph Sacco, and Lee Schwamm, and the most relevant authors were also summarized (Fig. 3A). Institutions such as Capital Medical University (Beijing, China) and Harvard Medical School (Cambridge, USA) served as key hubs in translational research collaboration (Fig. 3B). Chinese–U.S. collaborations accounted for more than 28% of all co-authored studies (Supplementary Fig. 1).

Thematic evolution and keyword clusters

Keyword co-occurrence analysis (Fig. 4) identified 3 major phases: 1. 2005–2012: Focused on “atherosclerosis,” “LDL,” and “acute ischemic stroke”. 2. 2013–2018: Expanded to include “diabetes,” “metabolic syndrome,” and “statin therapy”. 3. 2019–2024: Shifted toward “HDL dysfunction,” “machine learning,” and “COVID-19-related vascular inflammation”. Detailed keyword clusters and silhouette values are summarized in Supplementary Table 3. These thematic shifts reflect the maturation of the field from foundational pathological mechanisms toward individualized, data-driven prevention.

Meta-analytical findings

Study selection and characteristics

Of the 14,619 records retrieved, 13 eligible studies were included in both the qualitative synthesis and quantitative meta-analysis in the final analysis (Fig. 5A). Key study characteristics, including sample size, diagnostic criteria, and lipid parameters, are detailed in Supplementary Table 4.^{18–28} Most studies were hospital-based and involved adult patients with IS diagnosed using computed tomography/magnetic resonance imaging (CT/MRI) and the TOAST criteria (Supplementary Table 4). Quality assessment (Fig. 5B) indicated a low-to-moderate risk of bias across the evaluated domains.

Lipid abnormalities in ischemic stroke: Pooled results

Meta-analysis revealed significant differences in lipid levels between patients with IS and healthy controls (Fig. 6). The pooled mean difference (MD) for TC was 0.94 mmol/L

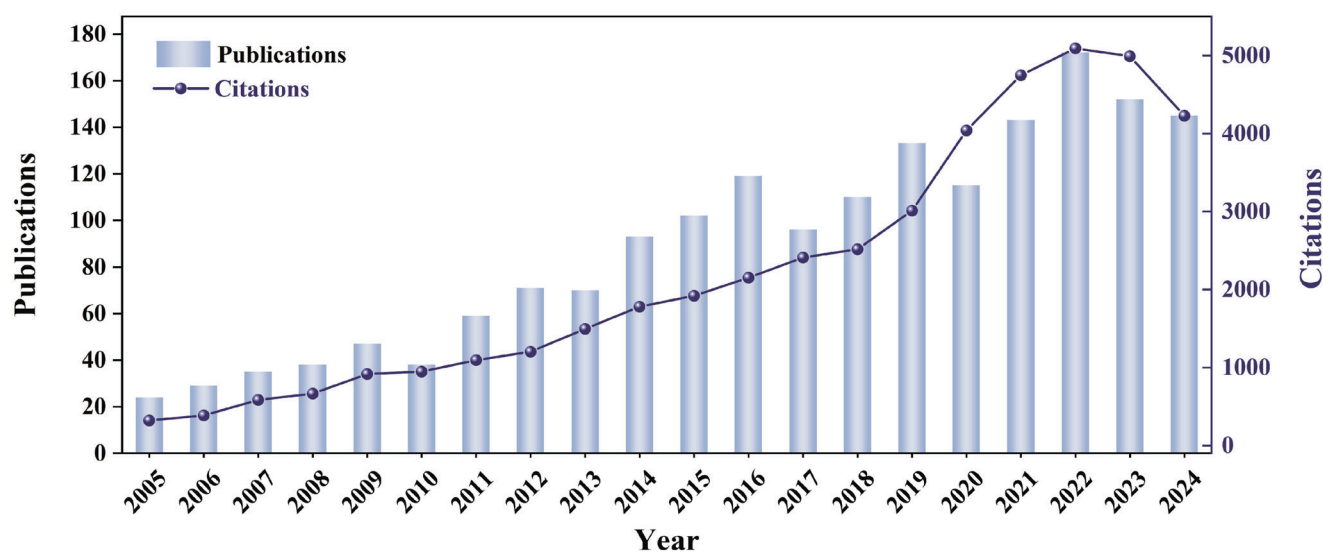


Fig. 2. Annual publication trends in dyslipidemia and ischemic stroke (IS) research from 2005 to 2024. Bars indicate the annual number of publications, and the line indicates citation counts

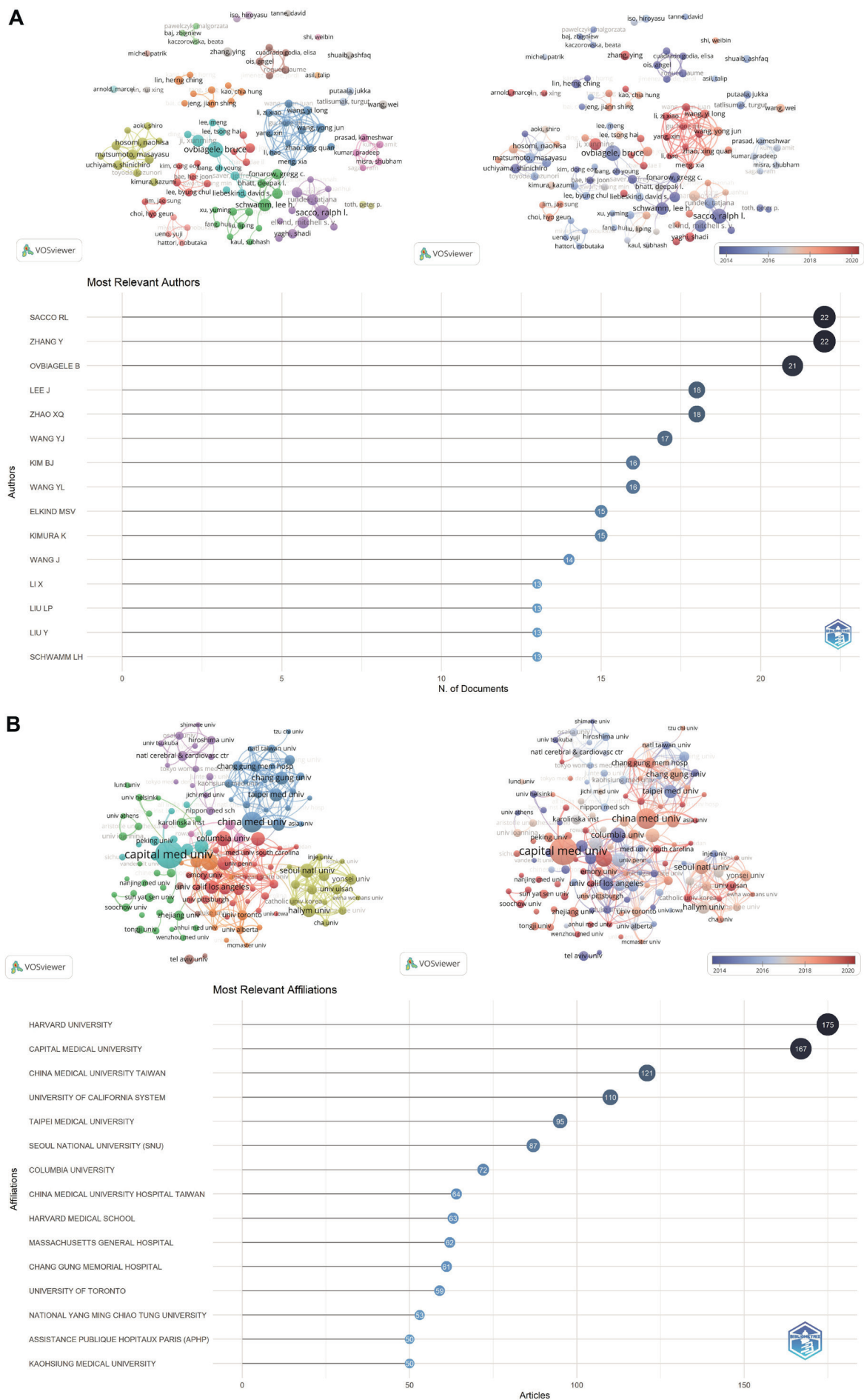


Fig. 3. Collaborative networks in dyslipidemia and ischemic stroke (IS) research. A. Author collaboration networks and publication patterns; B. Institutional collaboration networks and geographic distribution

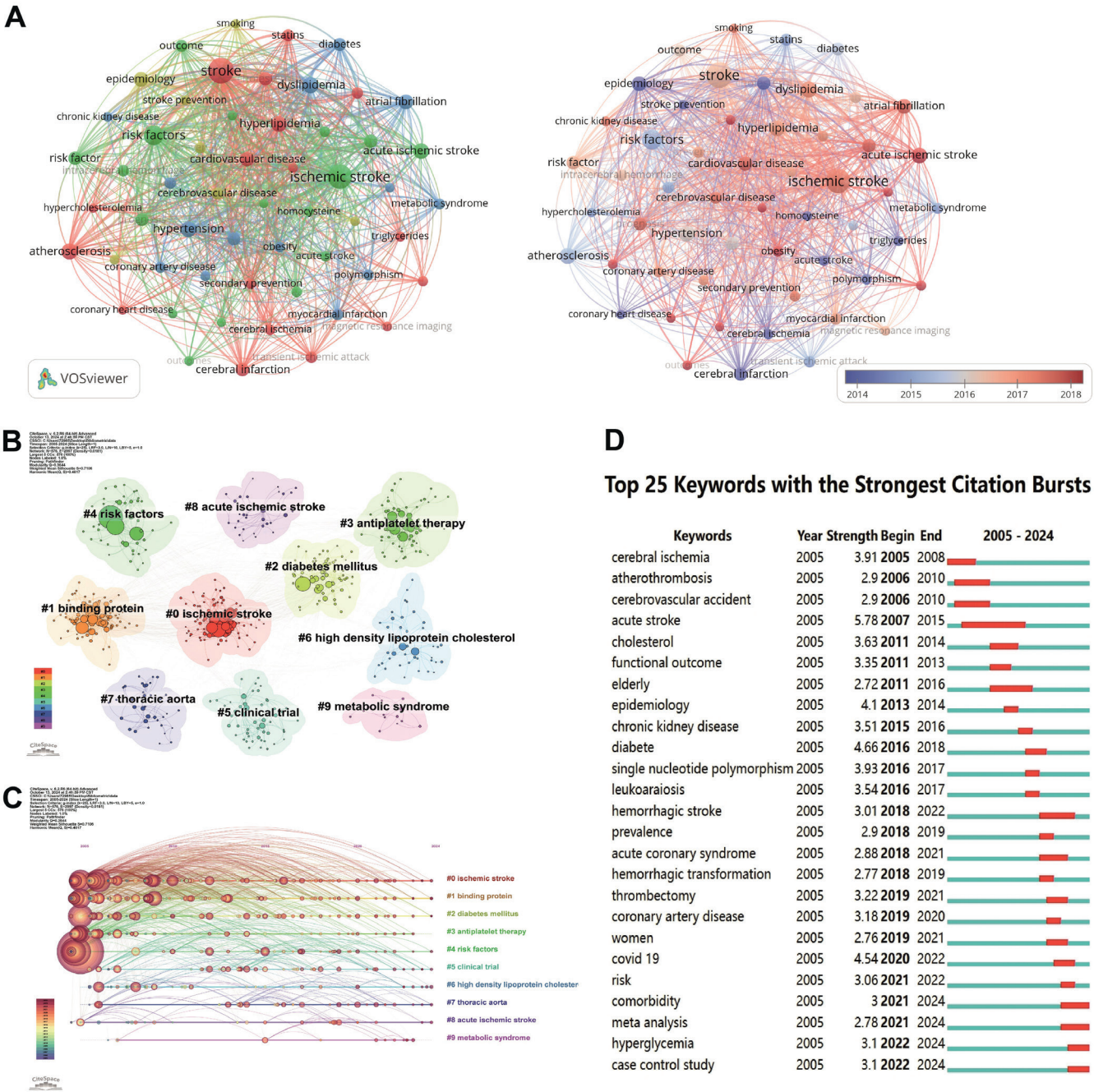


Fig. 4. Multidimensional evolution analysis and dynamic mapping of research trends in dyslipidemia and ischemic stroke (IS). A. Keyword co-occurrence networks; B. Keyword clustering analysis; C. Temporal evolution of keyword trends; D. Temporal patterns of emerging keywords

(95% CI: 0.36–1.52, $p < 0.001$), while TG levels were higher by 0.37 mmol/L (95% CI: 0.19–0.55, $p < 0.001$). Low-density lipoprotein cholesterol levels were elevated by 0.66 mmol/L (95% CI: 0.33–0.99, $p < 0.001$), whereas HDL-C levels were lower by –0.30 mmol/L (95% CI: –0.41 to –0.18, $p < 0.001$). Overall, these results demonstrate that dyslipidemia is significantly associated with an increased risk of IS, highlighting the importance of comprehensive lipid management in stroke prevention. Forest plots illustrating these findings are presented in Fig. 6.

Heterogeneity and effect modifiers

Substantial heterogeneity was observed in all analyses ($I^2 > 90\%$). Potential sources of heterogeneity mainly included ethnic variations and regional differences in baseline lipid profiles, differences in stroke subtypes (e.g., large-artery vs small-vessel disease), timing of lipid measurement (acute vs recovery phase), statin use, and comorbidity profiles. These confounding factors are likely contributors to between-study variability, although the direction of the observed effects remained consistent.

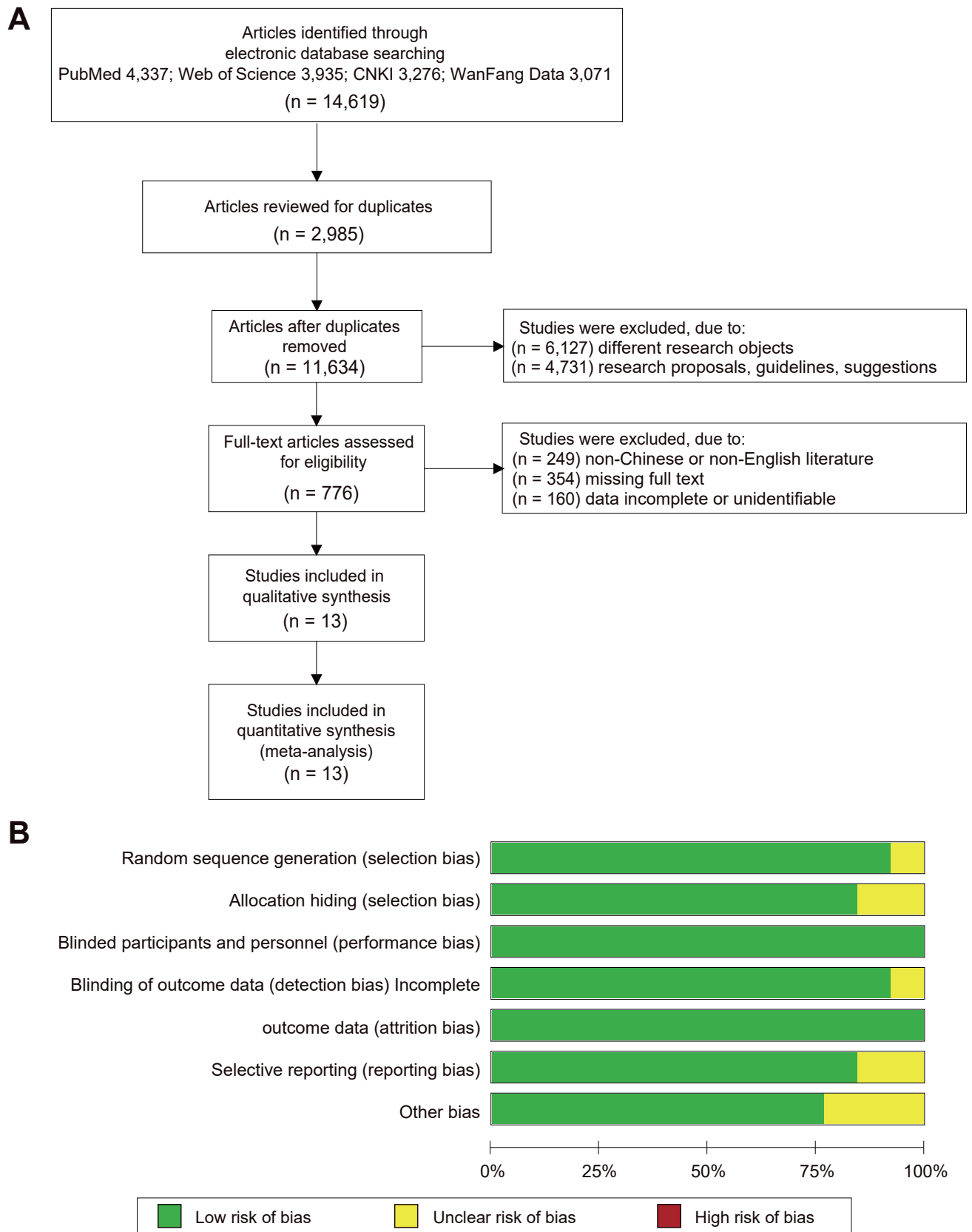


Fig. 5. Literature screening and quality assessment of the included studies. A. Flowchart of the study selection process; B. Summary of the risk-of-bias assessment for the included studies

Sensitivity analysis and publication bias

Sensitivity analyses (Fig. 7, Supplementary Tables 5–8) confirmed the robustness of the findings. Sequential exclusion of individual studies did not alter the direction or statistical significance of the pooled effect sizes. Publication bias was evaluated using funnel plots and Egger's regression test. Egger's test indicated no significant small-study effects for TC ($p = 0.120$), TG ($p = 0.170$), LDL-C ($p = 0.400$), or HDL-C ($p = 0.630$), which was consistent with the visual symmetry of the funnel plots (Fig. 8, Supplementary Table 9), suggesting a low risk of reporting bias.

Discussion

Clinical implications of pooled findings

This study confirms a strong association between dyslipidemia and the risk of IS. Specifically, elevated TC, TG, and LDL-C levels, together with decreased HDL-C levels, were consistently observed among patients with stroke. These findings are consistent with previous studies and reinforce the role of lipid abnormalities as independent and modifiable risk factors for IS.^{29,30}

From a clinical perspective, these findings support the incorporation of routine lipid monitoring, particularly HDL-C/LDL-C ratios, into stroke risk assessment. Elevated LDL-C and TG levels may promote atherosclerotic plaque formation and endothelial dysfunction, whereas reduced HDL-C levels may impair vascular repair mechanisms. These markers may help clinicians identify high-risk individuals at an earlier stage, thereby facilitating precision prevention strategies and targeted lipid-lowering interventions.^{31,32}

Methodological contributions: The dual framework

Unlike traditional single-method reviews, this study employed a dual approach integrating scientometric mapping and meta-analytic synthesis. Scientometric analysis revealed evolving research trajectories, from an early emphasis on atherosclerosis to an emerging focus on HDL dysfunction, lipidomics, and machine learning models. Author and institutional network analyses highlighted the growing prominence of China–USA collaborations and the emergence of translational research platforms.³²

The meta-analysis provided robust clinical evidence across diverse populations despite substantial heterogeneity. Sensitivity analyses confirmed the stability of the observed associations across all lipid indicators. Together, the 2 methodologies formed a closed-loop framework in which bibliometric insights informed clinical relevance and vice versa. This hybrid model may serve as a blueprint for future reviews, particularly in fields where the volume of research is large but fragmented.^{33,34}

Interpretation of heterogeneity

High between-study heterogeneity ($I^2 > 90\%$) was observed, particularly for TC and LDL-C. Several factors likely contributed to this heterogeneity, including population diversity (e.g., ethnicity and comorbid diabetes), variation in stroke subtypes (e.g., large-artery atherosclerosis vs small-vessel disease), timing of lipid measurement during the acute and recovery phases, and differences in medication use, particularly statins and traditional Chinese medicines. Although heterogeneity complicates effect estimation, it does not undermine the overall direction of the observed associations. Rather, it highlights the urgent need for subgroup-specific analyses and standardized reporting practices in future research.³⁵

Implications for future research and policy

Our findings highlight multiple actionable directions spanning clinical practice, research innovation, and policy development. In clinical practice, lipid screening should be prioritized in stroke risk models through the incorporation of lipid ratios and functional HDL subclasses. From the perspective of research innovation, multi-omics approaches, including lipidomics and metabolomics, combined with AI, may be leveraged to develop predictive tools for stroke occurrence in dyslipidemic populations. Policy development should encourage the integration of lipid targets into stroke prevention guidelines, particularly in regions with a rising prevalence of metabolic syndrome.

Future studies should emphasize multicenter cohorts, longitudinal follow-up, and data harmonization across different ethnic groups and healthcare systems. Linking lipid profiles with genetic polymorphisms, dietary patterns, and inflammatory biomarkers may provide deeper insight into the pathogenesis and prevention of IS.^{36,37} In recent years, women and hyperglycemia have emerged as important topics in stroke research. Evidence indicates that insulin resistance, hyperglycemia, and dyslipidemia in women with metabolic syndrome jointly increase the risk of atherosclerotic IS.³⁸ Findings from the China Health and Retirement Longitudinal Study further demonstrated that the atherogenic index of plasma and the TG-glucose index not only independently predict post-stroke all-cause mortality but also highlight the interactive effects of abnormalities in glucose and lipid metabolism on stroke prognosis.³⁹ Moreover, stress hyperglycemia has been shown to be strongly associated with poor outcomes in acute IS, particularly among non-diabetic patients.⁴⁰ Future research should therefore focus on the “women–hyperglycemia–dyslipidemia–stroke” axis to elucidate the underlying mechanisms and inform precision prevention strategies.

Limitations of the study

This study has several limitations that warrant consideration. We included only English- and Chinese-language

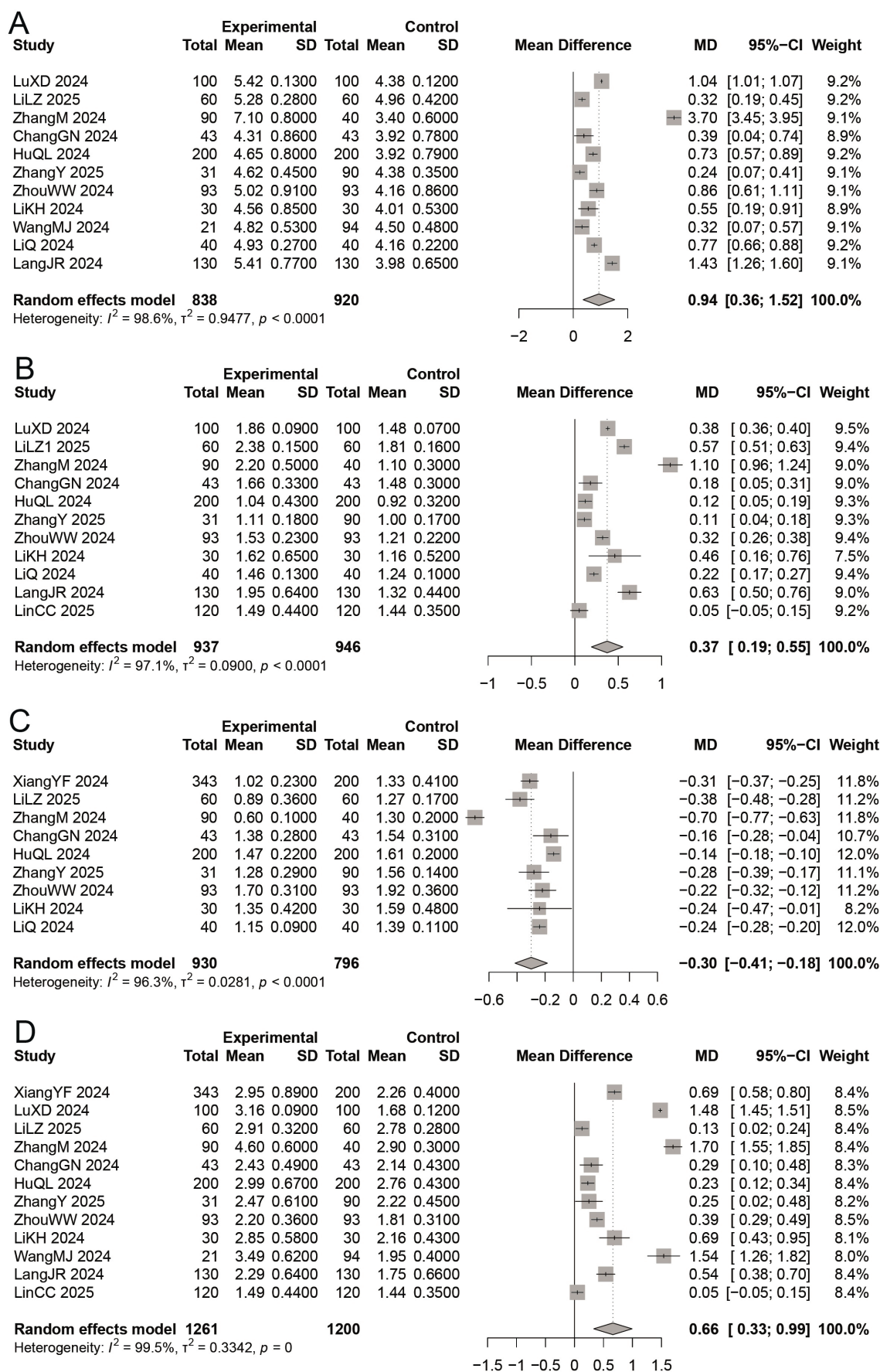


Fig. 6. Forest plots of the meta-analysis. A. Forest plot of the association between total cholesterol (TC) levels and ischemic stroke (IS); B. Forest plot of the association between triglyceride (TG) levels and IS; C. Forest plot of the association between high-density lipoprotein cholesterol (HDL-C) levels and IS; D. Forest plot of the association between low-density lipoprotein cholesterol (LDL-C) levels and IS

MD – mean difference; SD – standard deviation; 95% CI – 95% confidence interval.

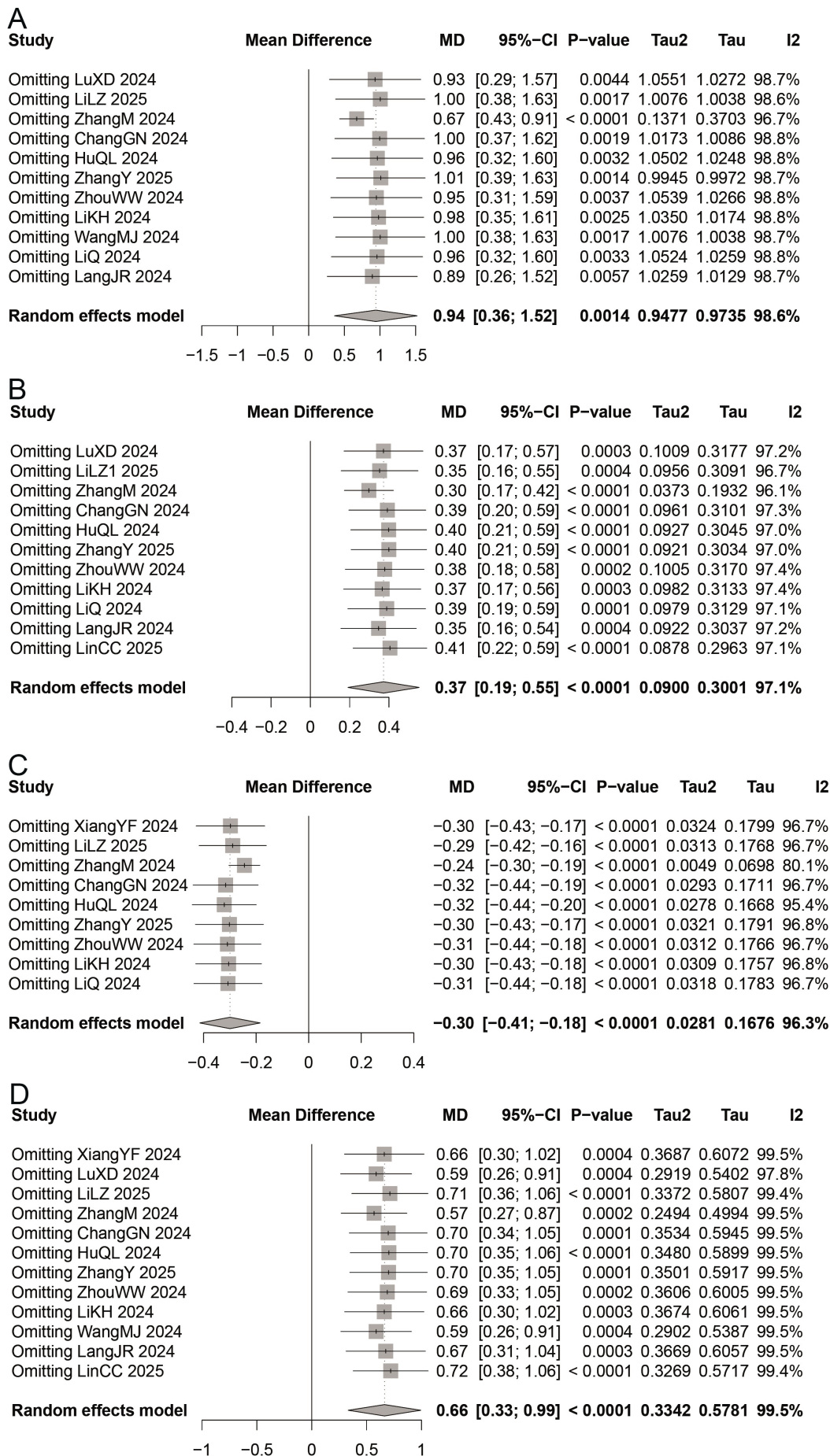


Fig. 7. Sensitivity analysis forest plots. A. Sensitivity analysis forest plot of the association between total cholesterol (TC) levels and ischemic stroke (IS); B. Sensitivity analysis forest plot of the association between triglyceride (TG) levels and IS; C. Sensitivity analysis forest plot of the association between high-density lipoprotein cholesterol (HDL-C) levels and IS; D. Sensitivity analysis forest plot of the association between low-density lipoprotein cholesterol (LDL-C) levels and IS

MD – mean difference; SD – standard deviation; 95% CI – 95% confidence interval.

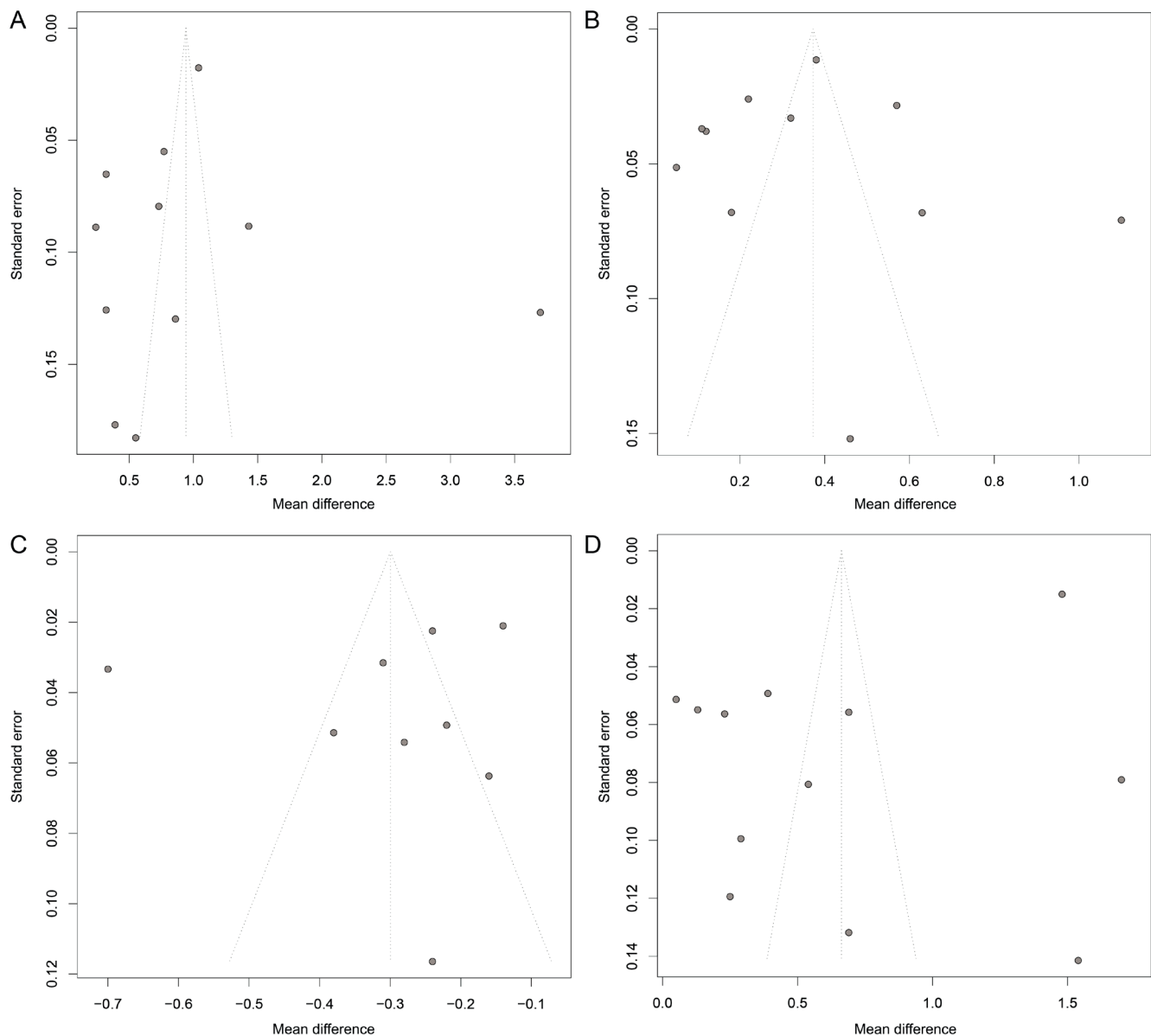


Fig. 8. Assessment of publication bias in the meta-analysis. A. Funnel plot of the association between total cholesterol (TC) levels and ischemic stroke (IS); B. Funnel plot of the association between triglyceride (TG) levels and IS; C. Funnel plot of the association between high-density lipoprotein cholesterol (HDL-C) levels and IS; D. Funnel plot of the association between low-density lipoprotein cholesterol (LDL-C) levels and IS

articles and relied heavily on the Web of Science and major databases such as PubMed and CNKI. This may have introduced selection bias, potentially excluding high-quality studies published in other languages or in regional journals not indexed in major databases. The meta-analysis relied on aggregate data from published studies rather than individual patient data (IPD). This limited our ability to conduct subgroup analyses based on age, sex, comorbidities (e.g., diabetes and hypertension), or stroke subtype (e.g., cardioembolic vs atherothrombotic). Despite consistent effect directions, high heterogeneity ($I^2 > 90\%$) was observed for all lipid markers. This heterogeneity likely reflects differences in population demographics, diagnostic criteria, measurement timing (acute vs recovery phase), and statin use. Although random effects models were used to account for this variability, the underlying heterogeneity may still

influence the generalizability of the pooled estimates. Most included studies were observational, introducing the potential for residual confounding and limiting causal inference. Few studies adjusted for all relevant vascular risk factors or used standardized lipid testing protocols. The analysis was limited to traditional lipid parameters (TC, TG, HDL-C, and LDL-C). Novel or functional markers, such as HDL particle size, oxidized LDL, and ApoB/ApoA1 ratios, were rarely reported and therefore could not be included, limiting mechanistic interpretation. These limitations highlight the need for future prospective, multicenter, IPD-based studies with standardized methodologies and broader database inclusion to validate and extend our findings.

The interpretation of keyword burst analysis also has inherent limitations. Some bursts may be driven by external events or editorial policies rather than by substantive shifts

in research activity. For instance, the surge in the keyword “COVID-19” reflects the temporary impact of a global health crisis rather than a fundamental transformation of the field of lipid–stroke research. Similarly, the emergence of terms such as “case-control study” and “meta-analysis” is partly attributable to journal requirements for specifying study design in titles or abstracts, rather than to a sudden increase in their intrinsic importance. Therefore, burst keywords should be interpreted with caution and in the context of publication practices, editorial standards, and broader scientific trends. Although our meta-analysis included studies involving various IS subtypes, data specific to lacunar infarction and other stroke subtypes were insufficient for separate analyses. This limitation prevents a comprehensive evaluation of the role of precision lipid management and personalized prevention in subtype-specific settings. Future studies should stratify patients according to stroke subtype, particularly lacunar infarction, to determine whether tailored lipid-lowering interventions can improve outcomes.

Conclusions

This study provides integrated evidence from scientometric and meta-analytic perspectives, confirming that dyslipidemia, characterized by elevated TC, TG, and LDL-C levels and reduced HDL-C levels, is significantly associated with an increased risk of IS. These findings support the routine use of lipid profiling, particularly HDL-C/LDL-C ratio monitoring, as part of risk stratification in both primary and secondary stroke prevention. Our dual-framework approach highlights not only clinical associations but also global research trends, revealing a shift toward precision lipid management and personalized interventions. Future research should focus on multicenter, multiethnic cohort studies, integration with lipidomics and AI-driven prediction models, and refinement of lipid-related guidelines for stroke prevention. This comprehensive strategy may accelerate the translation of lipid-marker research into real-world clinical tools that reduce the global burden of stroke.

Supplementary data

The supplementary materials are available at <https://doi.org/10.5281/zenodo.17628518>. The package contains the following files:

Supplementary Fig. 1. Multidimensional visualization of international collaboration networks and knowledge dissemination in dyslipidemia and IS research.

Supplementary Table 1. Top 20 productive authors and institutions in dyslipidemia and IS research (2005–2024).

Supplementary Table 2. Country-level research output and leading journals (2005–2024).

Supplementary Table 3. Detailed parameters of keyword clustering.

Supplementary Table 4. Characteristics of the included studies.

Supplementary Table 5. Sensitivity analysis of the association between TC level and IS.

Supplementary Table 6. Sensitivity analysis of the association between TG level and IS.

Supplementary Table 7. Sensitivity analysis of the association between HDL-C level and IS.

Supplementary Table 8. Sensitivity analysis of the association between LDL-C level and IS.

Supplementary Table 9. Egger’s test results for publication bias.

Use of AI and AI-assisted technologies

Not applicable.

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GPR149 (G protein-coupled receptor 149): Structure, signaling, and emerging roles in reproduction, metabolism, and neural function

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Abstract

G protein-coupled receptors (GPCRs) constitute one of the largest and most versatile families of membrane proteins, mediating a wide range of physiological functions through intracellular signaling pathways. Among them, G protein-coupled receptor 149 (*GPR149*) is an orphan receptor that has recently attracted increasing attention due to its distinctive structure and emerging biological roles. Although initially identified as an “antifertility” gene with high ovarian expression, accumulating evidence suggests a broader physiological relevance, including roles in metabolic and neurological regulation. This narrative review summarizes and integrates current evidence on the structure, expression patterns, and functional roles of *GPR149* across multiple biological systems, including reproduction, metabolism, and the central nervous system (CNS), while highlighting existing knowledge gaps and future research directions. *GPR149* appears to act as a negative regulator of fertility and ovulation, influencing oocyte maturation and granulosa cell signaling. In metabolic regulation, *GPR149* deficiency is associated with resistance to diet-induced obesity and improved insulin sensitivity. In the nervous system, *GPR149* expression in glial and neuronal populations has been linked to myelination and neuroendocrine signaling, primarily through modulation of the MAPK/ERK pathway. Altered expression of *GPR149* under diabetic and neurological conditions further supports its multifunctional role. Collectively, current evidence positions *GPR149* as a multifunctional orphan GPCR involved in the regulation of reproductive, metabolic, and neural processes. Elucidating its ligand interactions and signaling mechanisms may open new therapeutic avenues for reproductive disorders, metabolic syndromes, and neurodegenerative diseases.

Key words: signaling pathways, neural development, *GPR149*

Cite as

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Highlights

- GPR149 (G protein-coupled receptor 149) regulates reproduction, metabolism, and neural function.
- GPR149 signaling involves MAPK/ERK pathways and reproductive cell regulation.
- GPR149 negatively regulates fertility, ovulation, and oocyte maturation.
- GPR149 deficiency improves metabolic homeostasis and insulin sensitivity.
- GPR149 expression in the central nervous system links it to neural signaling and myelination.

Introduction

G protein-coupled receptors (GPCRs) are a large and diverse family of membrane proteins that play a key role in transmitting signals from outside to inside the cell, thereby regulating numerous physiological processes, including those in the nervous system.¹ They mediate a wide range of cellular responses to most neurotransmitters and hormones in the human body and exhibit extensive structural and functional diversity, enabling them to respond to various endogenous ligands.² Because of their crucial roles in maintaining cellular and tissue physiology and homeostasis, GPCR-related signaling pathways are implicated in the pathophysiology of numerous diseases, including metabolic, immune, and neurodegenerative disorders, as well as cancer and infectious diseases.^{3,4} G protein-coupled receptor 149 (*GPR149*) is a GPCR with a conserved C-terminal domain of unknown function and remains poorly characterized. *GPR149* encodes a seven-transmembrane receptor belonging to the class A GPCR family. Although classified as a class A GPCR, the protein lacks the first 2 charged amino acids of the highly conserved Asp-Arg-Tyr (DRY) motif in the 3rd transmembrane helix, which is critical for efficient G protein-mediated signal transduction.⁵

Computational analyses predict that *GPR149* is a class A rhodopsin-like GPCR consisting of 732 amino acids.^{6,7} Well-defined orthologs of this receptor have been identified in various vertebrate species, including mice and humans.⁸ *GPR149* possesses a highly conserved C-terminal domain of approx. 360 amino acids (in mice) that shows no similarity to other known proteins and may be involved in downstream signaling. However, its specific signaling pathways and endogenous ligands remain to be identified.⁶ *GPR149* has been detected in the dorsal root ganglion and gonads of chicken embryos,⁹ in the ventromedial hypothalamus of mice,¹⁰ and in astrocytes differentiated from a rat glial progenitor cell line.¹¹ *GPR149* may form dimers with other GPCRs expressed in oocytes, which could influence its function or cellular localization.¹² Its expression is low in newborn ovaries but gradually increases during folliculogenesis.⁸ *GPR149* has been identified as an “anti-fertility” gene expressed in the ovary and oocytes, making it of particular interest. It was discovered through database searches for genes with expression patterns similar to that

of *GDF9*.¹³ Using Gene Ontology (GO) classification, these genes were identified as GPCRs, among which *GPR3*, *GPR149*, and *GPR175* were highlighted as potentially important.^{14,15} Evidence indicates that *GPR149* deficiency does not alter the number of OL precursor cells but instead promotes their differentiation into OLs, leading to earlier myelination.⁷ It has also been reported that *GPR149* exhibits a distinct expression pattern in the kidney.¹⁶

Objectives

Despite being identified over a decade ago, *GPR149* remains one of the least characterized members of the GPCR family. Its unique structural features, including a modified DRY motif and a highly conserved C-terminal domain, together with its tissue-specific expression in the ovary, brain, and kidney, suggest specialized functions that have yet to be elucidated. Early studies indicate a potential role for *GPR149* in the regulation of ovarian folliculogenesis, OL differentiation, and myelination, highlighting its multifaceted physiological significance. However, to date, no narrative review has comprehensively summarized current knowledge on *GPR149*, including its structural characteristics, expression patterns, and potential biological roles. This review aims to synthesize existing evidence and provide insights into the unresolved aspects of *GPR149*, thereby offering a foundation for future functional and mechanistic studies.

GPR149 structure

The *GPR149* gene encodes a protein belonging to the class A family of 7-transmembrane GPCRs. *GPR149* contains a highly conserved C-terminal domain of approx. 360 amino acids that shows no similarity to other known proteins and may be involved in downstream signaling.⁶ This extended C-terminal region may play a role in receptor trafficking, intracellular signaling, or interactions with scaffold proteins such as β -arrestins. Similar extended C-terminal domains in other GPCRs have been implicated in receptor regulation and signaling bias. However, the specific signaling pathways and endogenous ligands of *GPR149* have not yet been identified. Phylogenetic

analysis of human GPCRs indicates that *GPR149* shares the greatest sequence similarity with receptors that bind peptide ligands.⁶ *GPR149* contains a modified DRY motif in the 3rd transmembrane helix, a sequence essential for efficient G protein-mediated signal transduction.¹⁷ Consequently, it is classified as a class A orphan GPCR.¹⁸ *GPR149*, also known as *PGR10* (progesterone-responsive gene 10) or *IEDA*, is a membrane protein composed of 731 amino acids (UniProtKB: Q86SP6; GP149_HUMAN) that spans the membrane multiple times. The absence of conserved Asp–Arg residues within the DRY motif of *GPR149* may affect its ability to efficiently couple to G proteins. Such variations have been associated with altered receptor activation, reduced G protein signaling, or a preference for non-canonical signaling pathways. *GPR149* functions as an orphan receptor and belongs to the GPCR family. The *GPR149* gene is located on human chromosome 3q25.2, a region that contains more than 1,100 genes, including a cluster of chemokine receptor (CKR) genes and several loci associated with human cancers.¹⁹ A key feature of *GPR149* is its unusually long C-terminal region, consisting of approx. 368 amino acids.^{15,18} Figure 1 illustrates the structure of *GPR149*.

GPR149 in reproductive system

Edson et al.⁸ conducted a study investigating the relationship between *GPR149* and fertility in a mouse model.

They found that *GPR149* is highly expressed in oocytes, with expression levels increasing during follicular development and decreasing after fertilization. The absence of *GPR149* leads to increased fertility and ovulation. *GPR149* was not expressed in granulosa cells but was highly expressed in germinal vesicle-stage and metaphase II oocytes. After fertilization, *GPR149* expression decreased and became undetectable by the 2-cell stage. To investigate the role of *GPR149* in oocyte growth and maturation, *GPR149* knockout mice were generated. These mice were viable and exhibited normal folliculogenesis but showed increased fertility, enhanced ovulation, elevated levels of oocyte growth differentiation factor 9 (GDF9) mRNA, and increased expression of follicle-stimulating hormone (FSH) receptor and cyclin D2 mRNA in granulosa cells.⁸ These findings suggest that *GPR149* may act as a negative regulator of fertility by suppressing pathways involved in granulosa cell proliferation and oocyte maturation. Another study reported that *GPR149* is associated with reproductive traits, demonstrating high haplotype similarity among Chinese breeds.²⁰ In mammals, *GPR149* is highly expressed in the ovary and brain, whereas knock-out mice exhibit increased fertility and enhanced ovulation.²¹ Notably, *GPR149* is also highly expressed in the pituitary gland of domestic chickens.²²

Thus, *GPR149* appears to play a regulatory role in reproductive physiology by acting as a negative modulator of fertility and ovulation. Its expression pattern – characterized by high levels in oocytes during follicular development

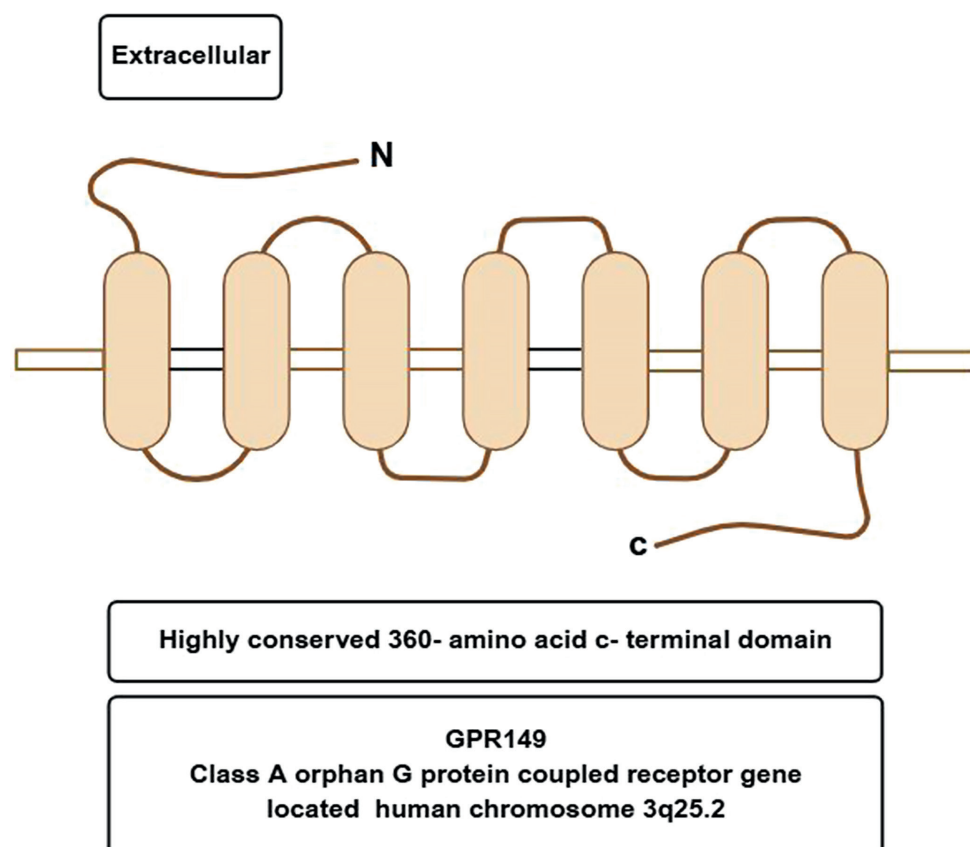


Fig. 1. Structure of G protein-coupled receptor 149 (*GPR149*): predicted seven-transmembrane topology highlighting key structural features, including the DRY (Asp–Arg–Tyr) motif variant and the extended C-terminal tail

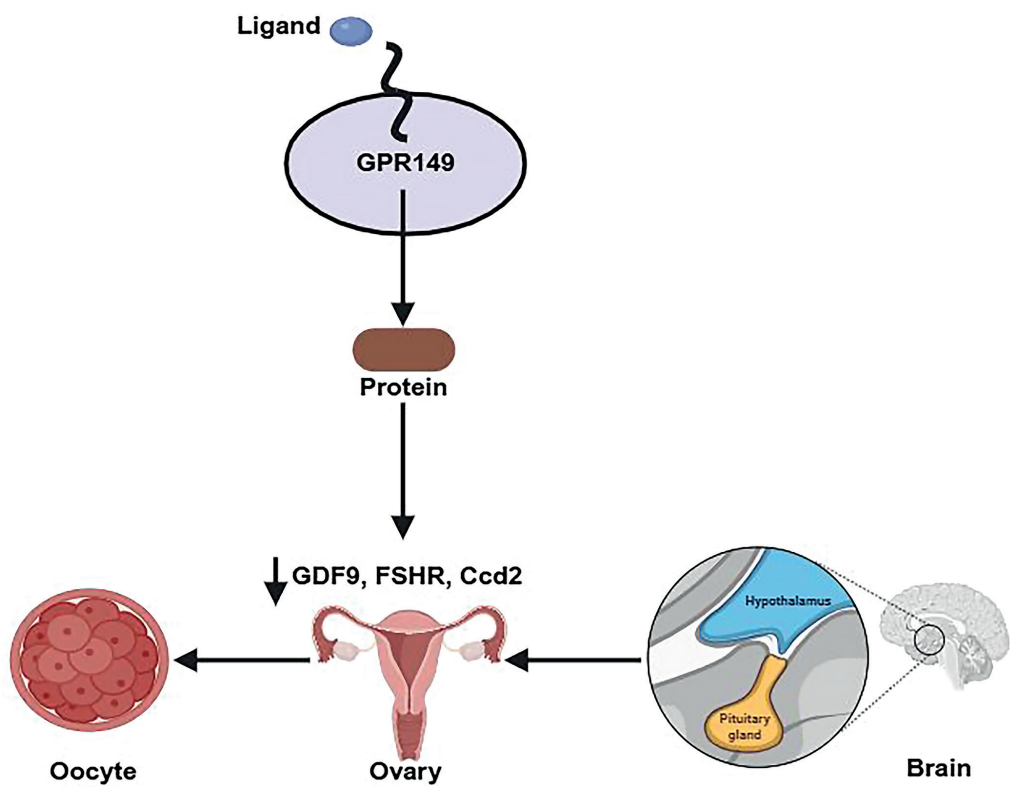


Fig. 2. Proposed role of G protein-coupled receptor 149 (*GPR149*) in the mammalian reproductive system: regulation of ovarian folliculogenesis, including negative modulation of ovulation and oocyte maturation inferred from knockout models

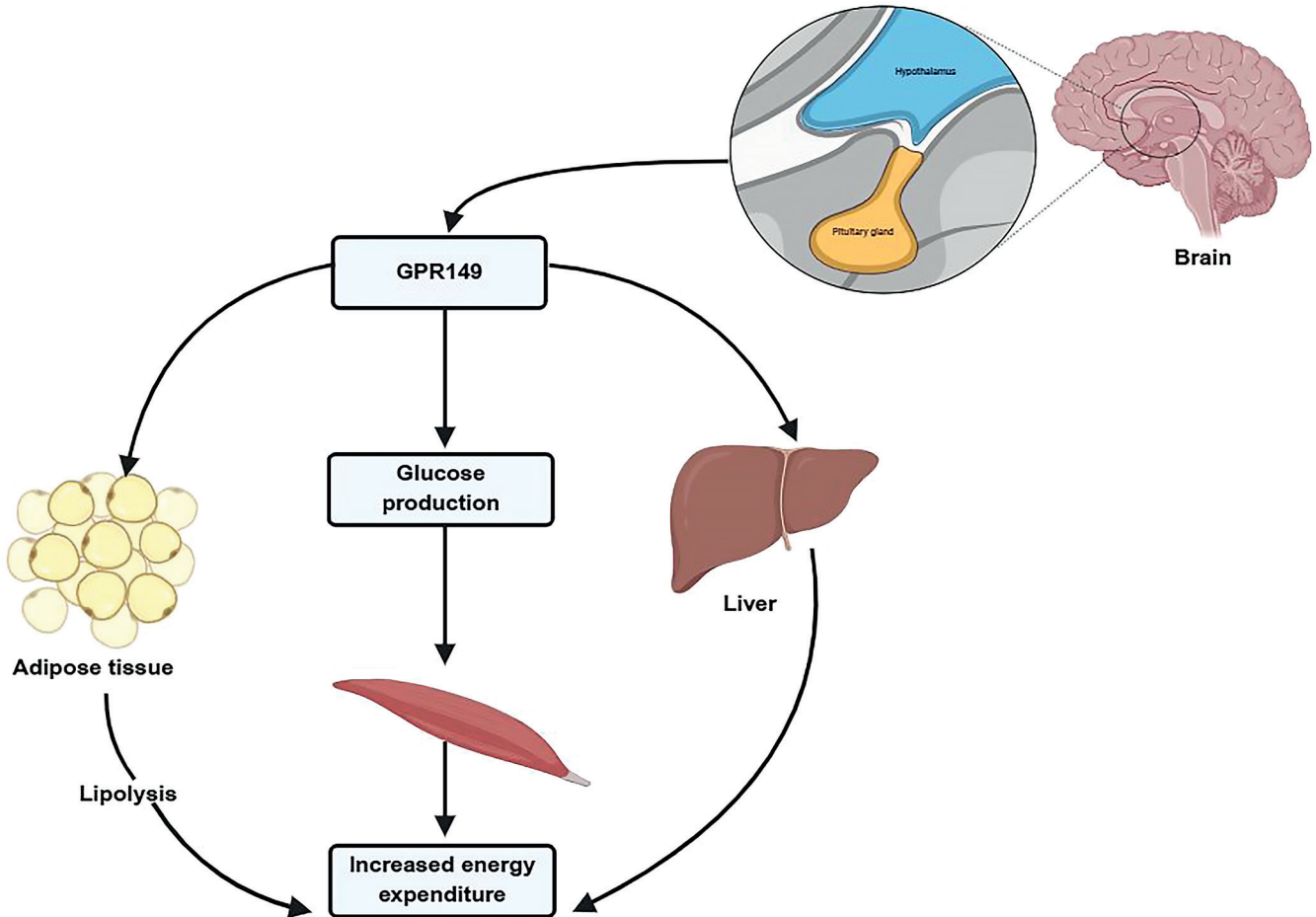


Fig. 3. Proposed role of G protein-coupled receptor 149 (*GPR149*) in metabolic regulation and energy homeostasis: hypothalamic pathways through which *GPR149* deficiency influences energy balance, including resistance to diet-induced obesity and improved insulin sensitivity

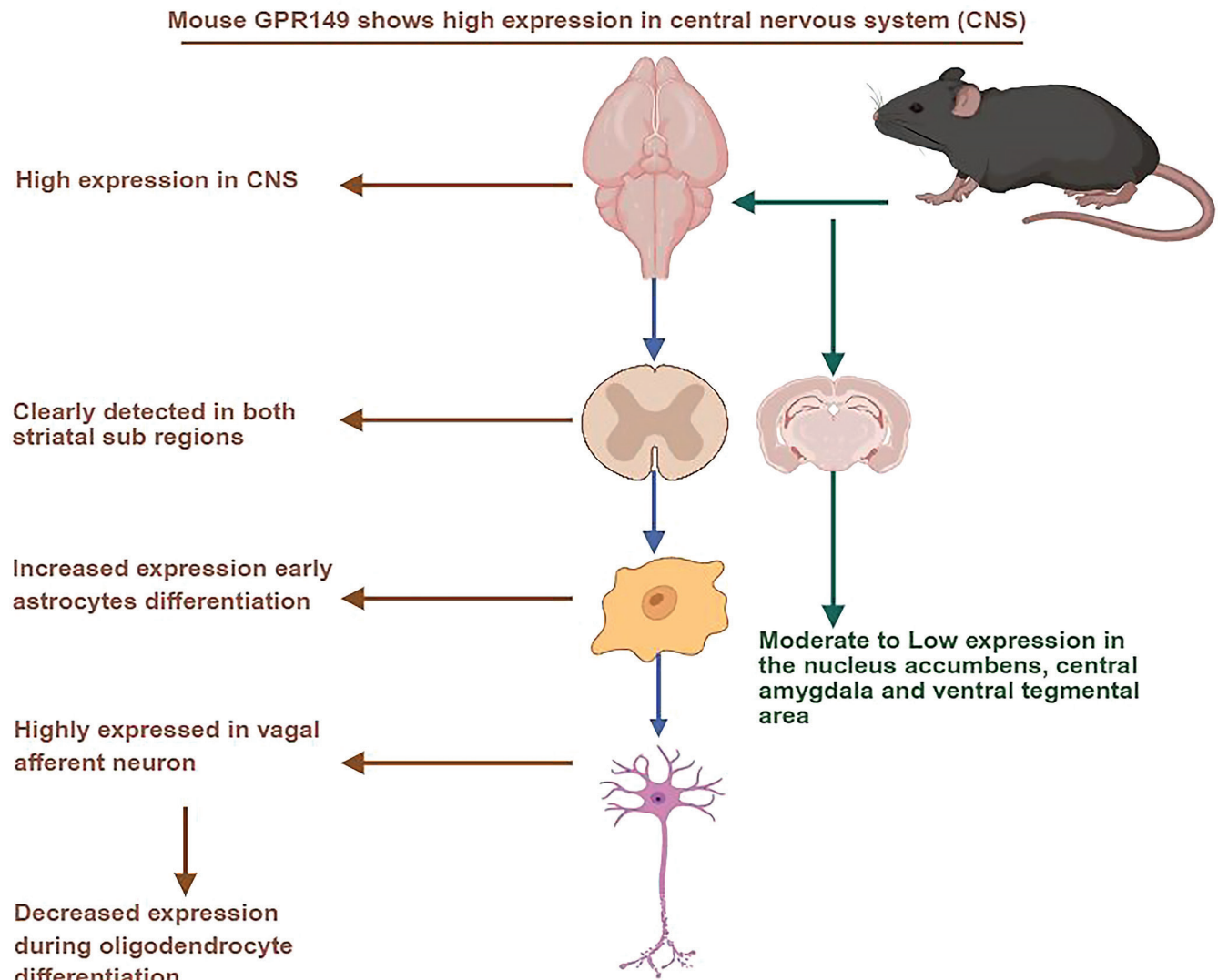


Fig. 4. Proposed mechanisms of G protein-coupled receptor 149 (*GPR149*) function in the central nervous system (CNS): putative signaling pathways, including regulation of the oligodendrocyte lineage via MAPK/ERK signaling and region-specific expression in basal forebrain structures

and a sharp decrease after fertilization – suggests a role in controlling oocyte maturation and early embryonic competence. Mechanistically, *GPR149* is likely to function through G protein-mediated signaling pathways to suppress downstream signaling molecules involved in follicular growth and hormone responsiveness. The increased expression of oocyte GDF9 follicle-stimulating hormone receptor (FSHR), and cyclin D2 (*Ccnd2*) mRNAs in *GPR149*-null mice indicates that *GPR149* normally inhibits pathways promoting granulosa cell proliferation and oocyte maturation.

These findings are consistent with previous studies demonstrating a regulatory role for *GPR3* and *GPR12* in spontaneous oocyte maturation.¹² In addition, the ability of *GPR149* to form dimers with other GPCRs may further modulate its signaling specificity, influencing receptor trafficking, ligand sensitivity, and downstream signaling. Although its endogenous ligand and precise signaling cascade remain unidentified, the conservation

of the C-terminal domain suggests an evolutionarily preserved regulatory mechanism, potentially involved in intracellular signaling, scaffolding, or receptor localization.

Notably, the distinct expression of *GPR149* in the pituitary gland of chickens suggests possible interspecies variation in its reproductive role, indicating that *GPR149* may integrate neuroendocrine cues with ovarian function and exhibit species-specific regulatory properties (Fig. 2). This observation warrants further comparative studies to determine whether the inhibitory effect of *GPR149* on fertility observed in mammals is conserved in avian species.

GPR149 in metabolism

Wyler et al.⁶ investigated the role of *GPR149* in metabolism and reported low levels of *GPR149* expression in the gastrointestinal tract. The highest expression levels were observed in the islands of Calleja and adjacent

nuclei, including the olfactory tubercle and the ventromedial hypothalamus. *GPR149*^{-/-} mice were viable and born at the expected Mendelian ratios, with no apparent abnormalities. The absence of hypothalamic *GPR149* expression in knockout mice was confirmed using quantitative polymerase chain reaction (qPCR). When fed a standard chow diet, *GPR149*^{-/-} mice showed a tendency toward slower weight gain. Upon switching to a high-fat diet (60% fat), these mice exhibited significantly reduced diet-induced obesity compared with wild-type controls, whereas *GPR149*^{+/-} mice displayed an intermediate phenotype. Furthermore, both *GPR149*^{-/-} and *GPR149*^{+/-} mice exhibited increased insulin sensitivity compared with control mice.

The authors concluded that *GPR149* remains classified as an orphan receptor, as its endogenous ligand(s) and downstream signaling pathways have not yet been identified. The findings suggest that *GPR149* acts as a regulator of energy balance, likely through central mechanisms involving the hypothalamus and other brain regions, possibly via Gi/o protein-mediated pathways affecting intracellular cAMP levels. *GPR149* has also been proposed as a potential therapeutic target for metabolic disorders, including diabetes and its complications, such as diabetic nephropathy.¹⁶ These conditions are associated with dysregulation of intracellular cAMP signaling pathways involved in renal cell function, inflammation, and fibrosis. In addition, reduced expression of *GPR149*, *GPR153*, and *GPR176* has been observed in the kidneys under diabetic conditions.¹⁶ Based on bioinformatic analyses, these receptors may couple to Gi/o proteins. *GPR149* may therefore influence renal function and metabolic complications by modulating Gi/o-dependent signaling, highlighting a potential link between central nervous system (CNS)-mediated metabolic regulation and peripheral organ function (Fig. 3).

This finding is significant, as alterations in Gi/o-mediated signaling have been implicated in key pathological mechanisms underlying diabetic nephropathy.¹⁶ Accordingly, reduced renal expression of these orphan Gi/o-coupled receptors in diabetes may contribute to the development and progression of diabetic nephropathy. Several G protein-coupled receptors, including *GPR149*, have also been reported to be differentially expressed in the habenular region.²³ This observation suggests that *GPR149* may modulate feeding behavior, reward circuits, or autonomic regulation, providing a broader central mechanism for its metabolic effects. Despite its initial classification as an orphan receptor, emerging evidence supports a role for *GPR149* in metabolic regulation. *GPR149* is expressed at relatively low levels in the gastrointestinal tract, while functional studies in knockout mice have shown that its deletion leads to reduced body weight gain and protection against diet-induced obesity. These findings indicate a role for *GPR149* in glucose metabolism and insulin signaling, potentially linking CNS control of energy balance with peripheral metabolic outcomes. These results are consistent with previous studies demonstrating roles for *GPR35* in adipose tissue energy homeostasis and inflammation,²⁴ and for *GPR12* in lipid metabolism.²⁵

GPR149 in central nervous system

Mouse *GPR149* is highly expressed in the CNS.⁸ It is strongly expressed in brain regions involved in energy regulation, including the ventromedial hypothalamus, olfactory tubercle, and islands of Calleja. *GPR149* shows moderate to low expression in the nucleus accumbens, central amygdala, and ventral tegmental area. It is also clearly detected in striatal subregions, including the caudate-putamen and nucleus accumbens.²⁶ This region-specific expression pattern suggests that *GPR149* may contribute to the integration of metabolic, emotional, and motivational signals within the CNS (Fig. 4).

GPR149 has been identified as one of the most highly expressed orphan receptors in vagal afferent neurons.²⁷ Its enrichment in vagal pathways suggests a potential role in gut–brain communication and autonomic regulation, both of which are central to energy balance and homeostasis. *GPR149* is also expressed at significantly higher levels in the brain than in other tissues.²⁸ A short cDNA fragment of *GPR149*, designated R35, was first identified through RNA fingerprinting in 1997.²⁹ Subsequently, the complete R35 sequence was obtained from chick embryonic day 8 (E8) dorsal root ganglia (DRGs), and its hydropathy profile revealed the characteristic structure of a seven-transmembrane receptor.⁹ This early identification in sensory ganglia supports a conserved role for *GPR149* in neural signaling.

GPR149 and *TRPM8* have been reported to be selectively enriched in peptidergic nociceptors in the DRG and trigeminal ganglia (TG). However, *GPR149* shows higher enrichment in DRG peptidergic nociceptors, whereas *TRPM8* is more highly expressed in TG peptidergic nociceptors.³⁰ This differential distribution suggests that *GPR149* may be more closely associated with spinal sensory processing than with cranial nociception. *GPR149* expression increases during the early stages of astrocyte differentiation but not during OL differentiation in the CG-4.B8 subclone of the rat glial precursor cell line CG-4.¹¹ Quantitative reverse transcription PCR (qRT-PCR) and double fluorescence in situ hybridization (dFISH) analyses have shown that *GPR149* is highly expressed in oligodendrocyte precursor cells (OPCs) and decreases during oligodendrocyte (OL) differentiation.⁷ This pattern supports the hypothesis that *GPR149* may function as an inhibitory regulator across multiple physiological systems, including neural and reproductive pathways. In addition, hypermethylation of the *GPR149* locus has been associated with shorter progression time in clear cell renal cell carcinoma, although further studies are required to establish its prognostic value.³¹

Deletion of *GPR149* in mice has been shown to result in transiently accelerated OL differentiation and increased myelination during development.⁷ Mechanistic studies indicate that *GPR149* constitutively activates the MAPK/ERK pathway, and inhibition of ERK signaling nearly

abolishes its suppressive effect on OPC differentiation into OLs. These findings suggest that *GPR149* regulates myelin development and regeneration through MAPK/ERK signaling. Overall, *GPR149* appears to act as a negative regulator of OPC differentiation, myelination, and remyelination. Targeting *GPR149* may therefore represent a promising therapeutic strategy to enhance myelin repair in demyelinating disorders.⁷

Several studies have shown that multiple orphan GPCRs are highly expressed in the mouse prefrontal cortex, a brain region associated with learning and memory.²⁶ *GPR149* has also been linked to neuroendocrine signaling and is prominently expressed in inhibitory interneurons within the ventromedial hypothalamus of mice. This localization suggests that *GPR149* may modulate inhibitory circuits involved in neuroendocrine and metabolic regulation. Moreover, genetic variants of *GPR149* have been identified in studies investigating susceptibility to migraine.^{28,32} The elevated expression of *GPR149* is particularly noteworthy given its association with inhibitory interneurons and myelination – processes often disrupted in neurological conditions such as amyotrophic lateral sclerosis (ALS), where excitotoxicity and myelination abnormalities have been observed.^{33,34} This overlap highlights *GPR149* as a potential modulator of neural stability and circuit integrity.

Notably, *GPR149* is expressed in several regions of the CNS, including the nucleus accumbens, central amygdala, ventral tegmental area, and striatal subregions such as the caudate-putamen. Its strong presence in vagal afferents and peptidergic nociceptors of the DRG suggests potential roles in sensory transmission and autonomic regulation.

In glial cells, *GPR149* expression changes dynamically during differentiation, increasing during early astrocyte development and decreasing as OPCs mature. Functional studies have shown that deletion of *GPR149* promotes OPC differentiation and accelerates myelination, indicating that the receptor acts as a negative regulator of myelin formation. Mechanistically, this effect appears to involve constitutive activation of the MAPK/ERK signaling pathway, as inhibition of ERK activity reverses the suppressive effect of *GPR149* on OL maturation. These findings highlight *GPR149* as a key modulator of myelin development and repair, with potential implications for demyelinating disorders. These results are consistent with previous studies demonstrating roles for *GPR37* in neurodegeneration and protein processing,³⁵ as well as in the regulation of OL differentiation and myelination.³⁶ Furthermore, its expression in inhibitory interneurons of the ventromedial hypothalamus, together with reported genetic associations with migraine, suggests additional roles in neuroendocrine signaling and sensory processing (Table 1).^{6–8,12,23–28,31} Despite these emerging insights, the endogenous ligand(s) and precise signaling mechanisms of *GPR149* remain unknown, underscoring the need for further mechanistic and translational studies targeting this orphan receptor.

Translational and therapeutic potential

GPR149 holds promise as a therapeutic target across reproductive, metabolic, and neurological systems. In reproduction, *GPR149*-null female mice exhibit hyperfertility, indicating that *GPR149* normally suppresses ovulation. Thus, its inhibition may enhance fertility in clinical or assisted reproductive technology (ART) settings, whereas its activation could support contraceptive strategies, although ligand identification remains necessary.^{8,37} Its oocyte-specific expression and pre-ovulatory peak suggest a modulatory role in gonadotropin responses. In addition, its potential as a biomarker of oocyte quality is supported by analogous findings in chickens related to reproductive timing.³⁸ Beyond reproduction, *GPR149* is expressed in brain regions associated with energy balance, and knockout studies demonstrate protection against diet-induced obesity and improved insulin sensitivity. These findings implicate *GPR149* in glucose regulation and position it as a potential target for the treatment of metabolic syndrome and type 2 diabetes.⁶ In the CNS, *GPR149* is highly expressed in OPCs and is downregulated during myelination. Deletion of *GPR149* enhances remyelination both in vitro and in vivo, suggesting therapeutic relevance in demyelinating diseases such as multiple sclerosis (MS).^{6,7}

Although no *GPR149*-targeted compounds are currently available, its cell type-specific expression supports its potential as a biomarker. In reproductive settings, *GPR149* levels may reflect ovulatory function, whereas in the nervous system, its downregulation during OPCs maturation may indicate remyelination.⁷ Single-cell RNA sequencing studies may further clarify its utility as a cell-state biomarker, particularly in rare neuronal populations such as those in the islands of Calleja.^{6,39} In summary, the selective expression and multifunctional roles of *GPR149* confer broad translational potential, ranging from therapeutic targeting in infertility, obesity, and MS to its future development as a biomarker in reproductive and neuroregenerative contexts.^{40,41}

Comparisons with other orphan GPCRs

GPR149's therapeutic potential is supported by successful examples of other orphan GPCRs. KISS1R (formerly GPR54), once considered an orphan receptor, became central to reproductive health following identification of its ligand, leading to the development of kisspeptin analogs now in clinical trials for pubertal and fertility disorders.³⁷ Similarly, *GPR17*, which is expressed in OPCs like *GPR149*, has emerged as a target in MS, with antagonists promoting remyelination and progressing to clinical trials.^{6,42} These findings suggest that *GPR149* could follow a comparable trajectory in neuroregenerative or reproductive therapies.

Table 1. Experimental evidence for *GPR149* across tissues and species

Species/tissue	Method (RNA-seq/qPCR/ISH/KO/GWAS)	Key finding (phenotype/readout)	Proposed mechanism/pathway	Reference
Mouse – metabolism (systemic)	Knockout (<i>GPR149</i> ^{-/-}); diet-induced obesity tests; glucose/insulin tolerance; indirect calorimetry; hypothalamic transcriptomics	In males, improved insulin sensitivity and resistance to diet-induced weight gain; altered energy homeostasis	Hypothalamic circuit effects (including VMH) consistent with energy-balance regulation	6
Mouse – CNS (oligodendroglial lineage: OPC/OL)	Cell-type data; signaling assays (ERK/MAPK readouts)	Evidence consistent with myelination/remyelination involvement; ERK/MAPK implicated	Hypothesized ERK-dependent regulation of OL differentiation / myelin repair	7
Mouse – ovary (oocyte/granulosa cells)	Knockout (<i>GPR149</i> ^{-/-}); fertility assays; ovarian histology; qPCR (cyclin D2)	Female <i>GPR149</i> ^{-/-} mice show increased fertility (larger litter size); ↑ Cyclin D2 mRNA in granulosa cells	<i>GPR149</i> acts as a negative regulator of ovulation / oocyte maturation	8
Mammalian – kidney (noted in the text)	Tissue expression datasets	Distinct expression pattern reported in kidney	Tissue-specific signaling (function to be elucidated)	12
Chicken – pituitary gland (adult)	Single-cell/atlas RNA-seq (domestic chicken)	High <i>GPR149</i> expression in pituitary	Possible role in endocrine regulation of reproduction	23
Mouse – hypothalamus (VMH and energy-balance nuclei)	Microarray/bulk RNA-seq mapping of nuclei	<i>GPR149</i> expression detected in energy-balance pathways	Putative participation in neuroendocrine control of energy balance	24
Mouse – striatum/limbic (islands of Calleja, nucleus accumbens)	Brain expression atlases (transcriptomic mapping)	High/region-enriched expression of <i>GPR149</i> in selected basal forebrain/striatal regions	Modulatory signaling in reward / limbic microcircuits (functional role to be defined)	25
Gut–brain/multi-tissue (contextual GPCR atlases)	Multi-omic atlases (RNA-seq; cell profiling)	<i>GPR149</i> present within broader GPCR landscapes intersecting metabolic circuits	Supports a systems view linking peripheral signals to CNS	26
Human – genome-wide association (neurological)	GWAS (migraine)	Risk-variant associations implicating <i>GPR149</i> locus in migraine biology	Genetic linkage to neuronal excitability / pain pathways (mechanism unresolved)	27, 31
Chicken embryo – dorsal root ganglion and gonads	In situ/developmental gene expression profiling	Detection of <i>GPR149</i> during embryonic development in sensory and gonadal tissues	Developmental role (conserved receptor expression; function to be tested)	28

GPCR – G protein-coupled receptor; CNS – central nervous system; RNA-seq – RNA sequencing; qPCR – quantitative polymerase chain reaction; ISH – in situ hybridization; KO – knockout; GWAS – genome-wide association study; VMH – ventromedial hypothalamus; OPC – oligodendrocyte precursor cell; OL – oligodendrocyte.

In metabolic disease, *GPR119* was orphanized as a lipid-sensing receptor, and several agonists have advanced to clinical trials for type 2 diabetes. Although early candidates showed limited efficacy, ongoing combination strategies underscore the importance of continued therapeutic development.^{43,44} A similar approach may be relevant for targeting *GPR149* in obesity. Orphan GPCRs have also shown promise in oncology; e.g., *GPR87* has been linked to cancer cell survival and is being explored as a potential therapeutic target.³⁹ Collectively, these examples illustrate that, once characterized, orphan GPCRs such as *GPR149* can become important clinical targets across diverse disease areas.

Limitations of the study

This narrative review provides an overview of the current knowledge on *GPR149*, integrating evidence from reproductive, metabolic, and neural studies. However, the lack of identified endogenous ligands and well-defined signaling mechanisms limits a comprehensive understanding of its physiological and pathological roles.

Ligand screening for orphan GPCRs relies on a combination of experimental and computational approaches. Traditional reverse pharmacology involves expressing receptors in cell-based systems and screening ligand libraries using signaling readouts such as cAMP or reporter assays, although its efficiency has declined over time.⁴⁰ High-throughput platforms, including β -arrestin recruitment assays (e.g., Tango and PRESTO-Tango) and luciferase-based reporters, have improved the detection of both basal and ligand-induced activity.³⁹ Many orphan GPCRs exhibit constitutive activity, enabling assessment of baseline signaling through the use of promiscuous G proteins or yeast-based systems, which can guide the selection of appropriate functional assays and the identification of inverse agonists.^{39,40} Structure-based approaches have gained increasing attention with the development of tools such as AlphaFold2 and cryo-electron microscopy (cryo-EM), which facilitate virtual ligand screening (VLS) by enabling receptor modeling and docking of large compound libraries, thereby streamlining candidate selection.³⁹ These advances have also revealed intrinsic agonists and bound lipids that may help infer endogenous ligands.⁴¹ Overall,

integrated in vitro and in silico strategies are increasingly used to deorphanize GPCRs. Further experimental and clinical studies are required to validate these approaches and clarify the therapeutic potential of *GPR149*.

Conclusions

GPR149 is a multifunctional orphan GPCR with important roles in reproduction, metabolism, and neural development. Current evidence highlights its function as a negative regulator of fertility, a modulator of energy balance and insulin sensitivity, and a contributor to myelination and neuroendocrine signaling. However, the absence of identified endogenous ligands and an incomplete understanding of its signaling pathways limit insights into its physiological and pathological roles. Future studies should focus on identifying *GPR149* ligands, elucidating its downstream signaling mechanisms, and exploring its function in human tissues. Sequence analyses indicate that *GPR149* is conserved across multiple mammalian species, suggesting an evolutionarily preserved function. Nevertheless, variations in specific regions, including the C-terminal domain and intracellular loops, may contribute to species-specific differences in receptor signaling and regulation. Further investigation of *GPR149* as a therapeutic target in reproductive disorders, metabolic diseases, and demyelinating or neurodegenerative conditions may provide novel avenues for clinical intervention.

Use of AI and AI-assisted technologies

Not applicable.

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