EABR
Experimental and Applied Biomedical Research
ISSN 2956-0454
eISSN 2956-2090
VOL 27 No 2
June, 2026

ISSN 2956-0454

Print
Faculty of Medical Sciences, University of Kragujevac

Indexed in
EMBASE/Excerpta Medica, Index Copernicus, BioMedWorld, KoBSON, SCIndeks, Chemical Abstracts Service, Cabell's Directory, Celdes, CNKI Scholar (China National Knowledge Infrastructure), CNPIEC, EBSCO Discovery Service, Elsevier - SCOPUS, Google Scholar, J-Gate, Naviga (Softweco), Primo Central (ExLibris), ReadCube, SCImago (SJR), Summon (Serials Solutions/ProQuest), TDOne (TDNet), WorldCat (OCLC)

Address:
Experimental and Applied Biomedical Research, Faculty of Medical Sciences,
University of Kragujevac 69 Svetozara Markovica Street, 34000 Kragujevac, PO Box 124, Serbia

http:/medf.kg.ac.rs/eabr
https://reference-global.com/journal/SJECR

EABR is published four times annually

Experimental and Applied Biomedical Research is categorized as a scientific journal of M51 category by the Ministry of Education, Science and Technological Development of the Republic of Serbia

CIP - Каталогизација у публикацији
Народна библиотека Србије, Београд

61

EABR : Experimental and Applied Biomedical Research / editor in chief Olgica Mihaljevic. - Vol. 27, no. 2 (june 2026)- . - Kragujevac : Faculty of Medical Sciences, University of Kragujevac, 2024- (Kragujevac : Faculty of Medical Sciences, University of Kragujevac). - 30 cm

Tromesečno. - Je nastavak: Serbian Journal of Experimental and Clinical Research = ISSN 1820-8665
ISSN 2956-0454 = EABR. Experimental and Applied Biomedical Research
COBISS.SR-ID 81208329

TABLE OF CONTENTS

Erratum

Document published with mistake in the title

Erratum to:
The Effects of Selective Serotonin Reuptake Inhibitors on Motility of Peripheral Smooth Muscles
Manojlović Katarina[1], Divjak Ana[1], Simanić Igor[2], Krstić Kristijan[1], Mladenović Kristina[1], Mijailović Sara S[3], Gogić Andjela D[3], Mladenović Rasa[4], Zdravković Nebojša D[3], Mitrović Kristina[5], Grbović Vesna[1]
DOI: 10.2478/eabr-2024-0011

Correct version should be:
Assessment of Functional Status and Activities of Daily Living in Patients After Lower Limb Amputation
Manojlović Katarina[1], Divjak Ana[1], Simanić Igor[2], Krstić Kristijan[1], Mladenović Kristina[1], Mijailović Sara S[3], Gogić Andjela D[3], Mladenović Rasa[4], Zdravković Nebojša D[3], Mitrović Kristina[5], Grbović Vesna[1]
DOI: 10.2478/eabr-2024-0024

Explanation:
The error occurred when the title from a previous paper was mistakenly published during the editing process.
The journal editors apologize for this mistake.

BARRIERS TO EFFECTIVE BREAST CANCER SCREENING PROGRAMS: A SCOPING REVIEW

Neda Milosavljevic [1,2], Marko Spasic [3,4], Ivana Ivanovic [5], Marija Zivkovic Radojevic [1,2], Bojan Stojanovic [3,4], Olivera Kostic [6], Petar Canovic [7] and Milos Grujic [1,5]

[1] *Clinic for Radiation Oncology, University Clinical Center Kragujevac, Kragujevac, Serbia*
[2] *University of Kragujevac, Faculty of Medical Sciences, Department of Clinical Oncology, Kragujevac, Serbia*
[3] *Clinic for General Surgery, University Clinical Center Kragujevac, Kragujevac, Serbia*
[4] *University of Kragujevac, Faculty of Medical Sciences, Department of Surgery, Kragujevac, Serbia*
[5] *University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia*
[6] *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia*
[7] *University of Kragujevac, Faculty of Medical Sciences, Department of Biochemistry, Kragujevac, Serbia*

Received: 28.01.2026.
Accepted: 28.03.2026.

Corresponding author:

Associate Professor Marko Spasic, MD, PhD, General and Breast Surgeon

Clinic for General Surgery, University Clinical Center Kragujevac, Kragujevac, Serbia
University of Kragujevac, Kragujevac, Faculty of Medical Sciences, Department of Surgery, Serbia
Svetozara Markovića Street No 69, Kragujevac, Serbia

E-mail: drmspasic@gmail.com

UDK:

Eabr 2026; 27(2):107-123
DOI:10.2478/eabr-2026-0011

ABSTRACT

Breast cancer screening is one of the most important secondary prevention strategy when effective, but participation rates varies across different health systems around the globe. Barriers to effective screening are multifactorial and overlapping. Using PRISMA-ScR, a scoping review was conducted, using PubMed/MEDLINE, Scopus, and Web of Science databases, related to breast cancer, screening programmes and barrier. Studies, published between 2000 and 2025, examining constrains for successful screening coverage, that met inclusion criteria were analysed, while diagnostic imaging, high risk population assessment and treatment/follow up strategies were excluded. From 2,262 initially included records, 103 studies were eligible – most of them originated from Europe 43.7%, then North America 34.0%, Asia–Pacific 20.4% while no studies were conducted exclusively in African continent. Analysis showed predomination of studies with quantitative designs - 68.9%. Almost half of studies were focused on organized screening (48.5%), 22.3% opportunistic screening, while others represented mixed/unclear contexts. Study analysis showed overlapping barriers across categories. The most commonly observed are patient-level barriers, in 75.7% of studies, followed by access-related (61.2%), health system/organizational (57.3%), socioeconomic and cultural (41.7%), and provider-related determinants (32.0%). Particular emphasis was on disadvantaged populations, such as low socioeconomic status women, ones that live in rural/remote communities, and/or women with disabilities, and those with previous false-positive screening reports. Indicating that addressing an isolated barrier is unlikely to lead to more effective screening; rather, actions should be directed toward integrated strategies that incorporate a personalized approach encompassing the identified barriers.

Keywords: *Breast cancer, mammography, screening programmes, barriers, participation, scoping review, health equity.*

INTRODUCTION

Breast cancer screening is one of the most effective tool for early disease detection and improved oncological outcomes. Mammography, as a gold standard is age specific recommended and international health initiatives emphasize the importance of organised, population-based approaches (1). However, programme effectiveness and real-world screening uptake vary significantly across health systems and settings. Previous reports have shown that access to screening and participation is influenced by a wide range of factors.

Regardless of widely available organized breast cancer screening programs in many countries, effective coverage rate remains a persistent challenge. European and international analyses point out that screening access and uptake are affected by a numerous constrains, such as (but not limited to) socioeconomic inequalities, geographic access, personal preferences, health system capacities (2).

The suboptimal screening participation underlying factors are multifactorial, describing multilevel barriers, identifying patient-centered determinants (fear, discomfort, cultural beliefs among others), systemic and health organizational determinants (infrastructure, costs, health care professional availability), and provider-related factors (such as communication and recommendation practices) (3). Those determinant may especially affect underserved groups, contributing to sustained disparities in screening uptake (4).

These barriers may be especially relevant for underserved, contributing to persistent inequalities in screening uptake. Although previous publications have explored screening participation determinants, current available evidence remains heterogeneous in terms of study design, context, populations under investigation, and a concise definitions of barriers. In addition, a number of published results come from high-income countries with long-established screening programmes, whereas low- and middle-income settings and vulnerable or underrepresented populations remain less thoroughly documented.

Evidence, obtained from real world data, indicate the significance of tailored interventions, such as educational approaches and screening system, to enhance breast cancer screening uptake, particularly in countries/populations without long standing, well organized screening programmes, as well as in resource-challenged systems (5).

Literature data is diverse and heterogeneous, in screening context, study design, ranging from interviews, surveys, observational analysis, mixed studies combining qualitative and qualitative approach to qualitative studies, surveys and implementation-focused work, accompanied with various definitions of barriers and screening successfulness. Additionally, quality assurance policies and standards, considering inadequate screening programme performance, emphasizing necessity for screening program and provider service redesign, as well as individualisation of approaches (6).

Given the complexity of the topic and the need for a broader mapping of the evidence, we conducted a scoping review to identify and categorize barriers affecting the effectiveness of breast cancer screening across different healthcare systems and populations.

The aim of this review was not only to summarize previously reported barriers, but also to provide findings relevant to programme improvement, policy development, and highlight potential interventions to improve screening uptake, particularly in settings where screening remains inadequate.

MATERIALS AND METHODS

Inclusion and exclusion criteria

Based on clearly defined, pre-established inclusion and exclusion criteria, studies aligned with the research objectives of this scoping review were selected. The selected publications included qualitative and quantitative research, as well as studies using combined methodologies, and addressed barriers, that is, factors determining the effectiveness of mammography screening for the purpose of early detection of breast cancer in different health systems.

The selected studies examined limitations encountered at different levels of the screening pathway, including the organization of the health care system, infrastructure, availability of services, and provider-related factors.

Barriers related to the target population itself (here, healthy women without known risk factors, screened with the aim of potential early detection of breast malignancy) were expanded to include psychological, socioeconomic, and cultural determinants. Geographic region or specific population characteristics were not considered exclusion criteria, nor were the pattern of screening program organization or national income level.

Studies were considered eligible if they were published as full-text articles, in English, in peer-reviewed scientific journals, within the period from 2000 to 2025. This timeframe was chosen to allow for monitoring the development of breast cancer screening programs, as well as for evaluating their effectiveness and identifying potential challenges to effective screening.

A summary of the eligibility criteria is presented in Table 1, while full publication details are provided in Supplementary material (Supplementary Table).

Studies with the exclusive aim of investigating diagnostic mammography or those focusing on the assessment of technical aspects were not considered. Meta-analyses, systematic reviews, narrative reviews, letters to the editor, editorials, conference abstracts, and case reports were not included in the analysis. In addition, populations with known risk factors (e.g., healthy BRCA-positive carriers), individuals with a

predefined high risk of developing breast cancer, as well as experimental and animal studies, were excluded. Studies that did not provide information relevant to the identification of barriers or factors influencing the effectiveness of breast cancer screening were also not considered. Incomplete or unavailable full-text articles were, among the aforementioned parameters, was considered as exclusion criteria.

Table 1. Eligibility criteria for study selection.

Domain	Inclusion criteria	Exclusion criteria
Study design	Original quantitative, qualitative, or mixed-methods studies	Reviews, meta-analyses, editorials, commentaries, conference abstracts, case reports
Population	Women eligible for or invited to mammography-based breast cancer screening in the general population or specific subgroups	Studies focused exclusively on high-risk genetic surveillance populations or symptomatic/diagnostic populations
Screening focus	Studies examining barriers, determinants, or facilitators related to participation in or effectiveness of mammography-based screening programmes	Studies focused exclusively on diagnostic mammography, imaging accuracy, or technical performance without behavioural, programmatic, or access-related context
Barriers / determinants of interest	Individual, socio-cultural, economic, health-system, provider-related, geographic, or informational barriers affecting screening uptake or effectiveness	Studies not addressing factors associated with screening participation, non-participation, or programme effectiveness
Geographic context	Studies from all geographic regions and income settings were eligible	No exclusions based solely on geographic region; however, studies outside the scope of organised or programme-based mammography screening were excluded
Publication characteristics	Peer-reviewed full-text articles published in English from 2000 onward	Non-English publications, unavailable/incomplete full-text articles, and studies published before 2000

Sources of Information

A comprehensive and systematic literature search was performed, in order to identify relevant eligible publications, in following databases: PubMed/MEDLINE**,** Scopus**,** and Web of Science, on 14 January 2026. Selection of this databases was due to extensive coverage of public health, biomedical and health services publications in per-review scientific journals.

The review protocol was prospectively registered on the Open Science Framework (OSF), in order to assure rigor in methodology and transparency - publicly available under the DOI https://doi.org/10.17605/OSF.IO/GCZJV, and complete project details can be accessed at https://osf.io/vqsc8.

Search Strategy

A systematic database search strategy was designed to identify relevant literature while accounting for potential variations in terminology, with the aim of recognise publications that examined factors influencing the effectiveness of breast cancer screening. Searches were conducted across databases using combinations of relevant keywords and Boolean operators, with a focus on studies addressing screening, access, and barriers.

Full texts were retrieved through institutional access provided by the Faculty of Medical Sciences, University of Kragujevac, Serbia, where available. Reference lists of relevant articles were also screened to identify additional eligible studies. Grey literature was not systematically searched and was not included in this review.

The following search string was used in aforementioned databases, with database-specific adaptations, where required: (“breast neoplasms” OR “breast cancer”) AND (“screening program*” OR “population-based screening” OR “organized screening” OR “mass screening” OR invitation OR registry) AND (barrier* OR determinant* OR uptake OR participation OR attendance OR nonattendance) NOT (diagnos* OR treatment OR therap* OR surviv*)

The asterisk (*) was used to denote truncation, allowing requisition of multiple word variants (e.g., therap* → therapy/therapeutic) and improving the sensitivity of the search strategy. Equivalent versions of this strategy were adjusted for PubMed/MEDLINE, Scopus, and Web of Science, regarding specificities in indexing systems and search

functionality. The full search strategies for each database are provided in the Supplementary Material.

Studies Selecting Process

Deduplication of publication was initial step in the process – obtained references were then exported, following importation into Zotero **(7)** for duplicate removal, performed on January**,** 15th 2026, prior to screening. The records remaining, were lather uploaded to Rayyan (8), in order to facilitate a transparent and reproducible workflow of the screening process (January**,** 17th 2026**)**.

Two independent reviewers (M.G.**,** and N.M.**)** screened titles and abstracts, respecting eligibility criteria, where potentially relevant records, by either reviewer, then accessed in full text, with subsequent confirmation of eligibility. If discrepancies occurred in any screening stage, and not resolved by consensus, a third reviewer (M.S.) was engaged to provide a final judgment.

The study selection process and reporting were conducted in accordance with the PRISMA Extension for Scoping Reviews (PRISMA-ScR) framework, maintaining transparent documentation of screening process.

Extraction of Data and Variables

Two reviewers were assigned to independently perform data extraction (M.G., and N.M.), using previously strandardised data-charting form, aiming to identify essential attributes within included studies.

Extracted data are presented in Supplementary Table and relate to study characteristics, including the first author's name, year of publication, country, study design, and screening context. When a target population was defined, it was recorded separately, including information on population subgroups where it was explored (e.g., minority groups, rural populations, and persons with disabilities). Characteristics of screening programs, indicators of screening performance, and measures of access were also among the extracted data.

Barriers to effective screening were documented as reported by the study authors and subsequently grouped into predefined thematic domains, including barriers related to screening participants (i.e., the target population), cultural and socioeconomic barriers, access-related barriers, and barriers related to organizational capacity at the health system level. When selected publications addressed multiple thematic domains, all relevant barriers were recorded without classification. Where available, potential recommendations or guidance for program improvement were also extracted and presented.

Given the objectives of this scoping review, as well as the heterogeneity of the included studies and terminology used, no additional outcome classification, pooling of estimates, or reanalysis of data was performed. Data were extracted as presented in the original studies, in line with the aim of mapping existing evidence without assessing effectiveness or introducing performance indicators.

Data Synthesis and Grouping of Evidence

In accordance with the study objectives, data synthesis was conducted using a structured narrative approach. Given the diversity of the extracted data, a descriptive presentation was used rather than a quantitative synthesis.

Studies were grouped according to key thematic domains, encompassing study characteristics and screening context, target populations, and, where reported, indicators of screening effectiveness. Identified barriers were categorically defined where appropriate; when studies pointed to the expected multifactorial nature of barriers to successful screening, all relevant thematic domains were included.

A quantitative synthesis was not attempted because of substantial heterogeneity across the included studies in terms of design, target populations, screening contexts, definitions of barriers, and outcome reporting, which did not allow meaningful result pooling Therefore, narrative synthesis was considered the most appropriate method for mapping and interpreting the available evidence for defining screening barriers.

RESULTS

Study selection

A total of 2,262 records were identified in literature search, across PubMed, Scopus, and Web of Science. Following 762 duplicates removal, 1,500 unique records were screened based on titles and abstracts and 1,102 records were excluded, due to failing to meet eligibility criteria.

Full texts were sought for 398 records, where 32 of them could not be retrieved. The remaining 366 articles were then assessed at the full-text level. Additionally, 263 articles were removed from consideration, due to failure to report barriers for screening or program effectiveness, or due to treatment focus, or limited to predefined risk population.

All eligibility criteria met 103 studies and included in the scoping review. The study selection process is summarized in the PRISMA flow diagram (Figure 1).

Included studies characteristics

Although review included studies published between 2000 and 2025, encompassing more than two decades of research, most studies were published after 2010, with 46 studies (44.7%) published between 2010 and 2019 and a further 21 studies (20.4%) published from 2020 to 2025. The median year of publication was 2012, reflecting growing focus on efficacy, participation and equity-related factors. An overview of the all included study characteristics, including publication period, geographic distribution, study design, screening context, and target population, is summarised in Table 2.

Table 2. Characteristics of included studies (n = 103)

Characteristic	n (%)
Publication period	
2000–2009	36 (35.0%)
2010–2019	46 (44.7%)
≥2020	21 (20.4%)
Geographic region	
Europe	45 (43.7%)
North America	35 (34.0%)
Asia-Pacific	21 (20.4%)
Africa	0 (0.0%)
Latin America	1 (1.0%)
Multinational	1 (1.0%)
Study design	
Quantitative	71 (68.9%)
Qualitative	21 (20.4%)
Mixed-methods	11 (10.7%)
Screening context	
Organized	50 (48.5%)
Opportunistic	23 (22.3%)
Mixed / unclear	30 (29.1%)
Target population (primary focus)	
General population	80 (77.7%)
Low socieconomic status	3 (2.9%)
Migrant / ethnic minorities	14 (13.6%)
Rural / remote	2 (1.9%)
Other	4 (3.9%)

As expected, study data were predominantly obtained from high-income countries. Europe accounted for the largest proportion of studies (43.7%), followed by North America (34.0%). A further 20.4% of studies were conducted in the Asia–Pacific region, while only a small number originated from Latin America (1.0%) or multinational settings (1.0%). None of analysed studies were conducted exclusively in African continent. Reflecting well established and organized screening programs, most frequently represented countries were United States of America, England, the Netherlands, Denmark, Sweden, Italy, and Australia.

The review included studies with a heterogonous design, with domination of quantitative methodology as much as 71 studies (68.9%), included cross-sectional surveys, registry-based analyses, spatial or ecological studies, and observational cohort designs. Qualitative methodologies were used in 21 studies (20.4%), via focus group interviews, exploring women's perceptions, and decision-making process. Mixed-method studies occupied 10.7% of all studies combining quantitative data with qualitative insights.

In terms of screening context, 48.5% included studies focused primarily on organized, screening programmes in age specific population and centralized program oversight. Majority of this studies investigated failure to respond to screening invitation, participation coverage in different socioeconomic and geographic groups. Opposed to organized screening, opportunistic screening was elaborated in of 22.3% of studies, reflecting health based systems and provider recommendation. Almost third of included studies (29.1%) addressed mixed or unclear screening contexts, in terms of comparing organized and opportunistic approaches.

Majority of studies (77.7%) targeted age specific population, but a notable subset examined specific, disadvantage populations, where barriers for effective screening are expected to be more pronounced, such as migrant or ethnic minorities (13.6%), women of low socioeconomic status (2.9%), and rural and/or remote populations (1.9%). A smaller group of studies (3.9%) addressed other specific subgroups, such as women with false-positive screening results or women with disabilities, reflecting socioeconomic and cultural inequalities in screening participation.

Failure to respond to screening invitation or non-attendance was exploited among various study designs, without unique, widely acceptable definition, where qualitative studies defined as non-attendance within a specified interval, focused on perceived barriers and prior experiences, other studies distinguished between women that never participated in screening, or the ones that undergo screened outside organized programs.

Barriers to effective breast cancer screening

Analysing included studies, barriers to effective breast cancer screening were observed covering multiple, frequently interrelated domains (Table 3), accentuating multifactorial nature of screening coverage – identified barriers at more than one level, rather than a single determinant. Representative examples of commonly reported barriers within domains are shown in Table 4.

The most frequently were patient-centered barriers, identified in 78 out of 103 studies, including fear of pain during mammography, fear of anticipated diagnosis or oncological treatment, anxiety due to radiation exposure, and low perceived personal risk of breast cancer, particularly among younger, asymptomatic women. Emotionally conditioned responses (fear, anxiety and fatalism) were emphasized, as expected, qualitative based studies related to previous negative experiences, such as pain and discomfort or a spectrum of negative emotional responses due to false-positive results. Growing personal and professional needs and limitations, accompanied comorbidities were among frequent reasons for postponed or canceled screening.

Table 3. Distribution of reported barriers to breast cancer screening across included studies (n = 103)

Barrier domain	Studies reporting barrier, n (%)
Patient-level barriers	78 (75.7%)
Access-related barriers	63 (61.2%)
Health system / organizational barriers	59 (57.3%)
Socioeconomic and cultural barriers	43 (41.7%)
Provider-related barriers	33 (32.0%)

Table 4. Key barriers to breast cancer screening reported across included studies

Barrier domain	Key barriers (representative examples)
Patient-level barriers	Fear of pain during mammography; fear of cancer diagnosis or treatment; low perceived personal risk; absence of symptoms; anxiety related to radiation exposure; previous negative screening experiences; competing life priorities (work, caregiving, illness).
Socioeconomic and cultural barriers	Low income; lack of health insurance or underinsurance; indirect costs (transport, time off work); low educational attainment; limited health literacy; social deprivation; financial insecurity despite availability of free screening.
Access-related barriers	Long distance to screening facilities; transportation difficulties; inconvenient appointment times; limited service availability; long waiting times; poor physical accessibility for women with disabilities; urban–rural disparities in service provision.
Health system / organizational barriers	Failure or delay in invitation letters; inaccurate population registries; lack of reminder or recall systems; fragmented care pathways; non-personalized screening processes; insufficient screening capacity; long screening intervals; administrative complexity.
Provider-related barriers	Absence of physician recommendation; inadequate communication about screening benefits and procedures; limited counseling time; inconsistent or unclear screening guidance; lack of cultural competence; language barriers during clinical encounters.

Socioeconomic barriers were observed in 41.7% and frequently overlapping with patient-centered factors, which are often not clearly distinguishable. Lack of financial stability or low income, lack of health insurance as well as and indirect costs of screening process (e.g. transportation, time off work, unpaid leave) were often seen as a barrier to screening participation emphasizing role of financial constrains despite the absence of direct screening costs. Cultural and social factors were particularly underlined across the studies, focusing on subpopulations such as migrant or ethnic minorities, where language barriers, compromised health literacy, cultural perception of breast cancer and preventive care, modesty concerns, and questionable trust of healthcare systems contributed to inadequate screening coverage rate.

Access-related barriers were reported in large number of the studies (61.2%) as one of most consistently identified structural barrier to effective breast cancer screening, where the most common emphasized obstacles are distance to medical facilities, transport related issues and limited service availability and they are especially pronounced in rural or remote. Even in urban or well-resourced health systems, number of studies showed that inappropriate scheduling, long waiting times or access related difficulties for women with disabilities will negatively affect participation in screening process, suggesting that access related barriers can have variable effect, depending on personal preference or limitations.

Health system and organizational barriers were described in 57.3% of studies, especially prominent in studies related to organized screening programs. Frequently reported issues refers to failures or delays in invitation letters, inaccurate population registries, absence of reminder or recall systems, and uninformed, organised screening pathways. Missed invitations, lack of follow up, long screening interval, lack of personalization or administrative errors or complex procedures were investigated in several studies.

Provider-related barriers were reported in 32.0% of analysed studies. Inadequate communication, inadequacy of clear recommendations for screening, as well as limited consultation time, were among reported barrier in this domain. Besides the aforementioned factors, language barrier can additionally contribute to ineffective screening in disadvantage populations.

DISCUSSION

Breast cancer is the most frequently diagnosed malignant tumour among women worldwide (9). Trends observed over the two past decades demonstrate a consistent increase in breast cancer incidence in many regions accompanied by declining mortality rates in high-income countries (10, 11). Projections for the next two decades show a substantial rise in breast cancer incidence worldwide, with region-specific increases across continents (12).

The economic burden of breast cancer treatment varies substantially and is highly dependent on disease stage, with annual health-care costs ranging from approximately $15,600 for early stage to $137,300 for stage IV disease (13).

Breast cancer represents one of the malignancies for which a clearly defined screening strategy exists, constituting a form of secondary prevention aimed at early disease detection (14).

Mammography is a broadly available and effective imaging modality that enables visualization of breast tissue abnormalities (15) and is widely used in countries with organized screening programmes as the primary method for early detection of suspicious breast lesions (16). Countries with established breast screening programmes have experienced decline breast cancer mortality compared with those without regular screening, emphasising the impact of organised breast cancer screening (17) Most of the high-income countries in Europe, North America and Asia-Pacific region have implemented organized screening programmes (1) while, some developed countries, including Greece and Bulgaria, did *not* have a fully implemented national organised mammography screening programme as of 2022 (18).

Participation rates in breast cancer screening vary considerably, ranging from less than 30% in countries such as Turkey to over 80 % in countries with well organized, well-established organized screening programmes, such as Finland and Netherland (18, 19). The reasons for inadequate participation in screening mammography are multifactorial and may be related to individual-level factors, accessibility, health system organization, and other structural barriers (20). Identifying and analysing these limitations, with the aim of proposing potential strategies for improving breast cancer screening programmes, constitutes the primary objective of this narrative review.

Topic of this scoping review was addressed because barriers to breast cancer screening cannot be predominantly attributed to a single domain, such as the patient or the health care system alone. Rather, an individual's decision to participate in screening is predominantly multifactorial, resulting from a complex interplay of personal, social, organizational, and structural determinants. Contemporary publications have largely focused on screening failure within specific populations defined by single determinant, wether young age, socioeconomic status, race, ethnicity ot other. However, although disparities across these groups are well documented, the underlying barriers to screening are inherently complex and frequently overpass and overlape beetwen categories. As a result, breast cancer screening remains insufficiently effective across a broad range of settings and populations, even in countries with established screening programmes (19). In this context, we conducted a scoping review to comprehensively map the existing evidence on barriers to breast cancer screening. A total of 103 studies, published beetwen 2000. and 2025. were included following a structured selection process involving systematic database searching, shown in PRISMA flow diagram (Figure 1).

Since eligible studies were published between 2000 and 2025, certain relevant barriers in identified earlier publications may not have been recognised. Interventions to improve screening uptake were not a primary focus of this review; therefore, studies describing such interventions were included only when detailed information was provided, and their selection was not systematic.

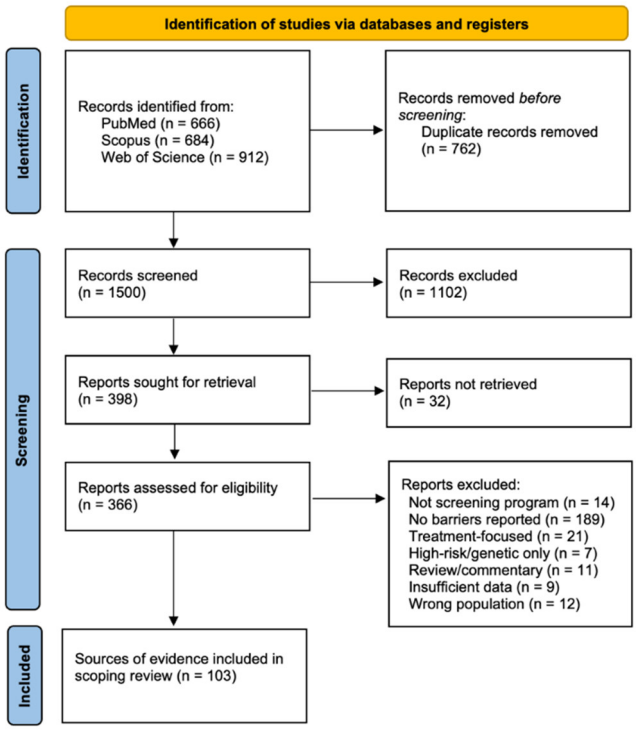
The most of included publications were published between 2010 and 2019, a period following the implementation and maturation of organized screening programmes in many countries. This timeframe allowed researchers sufficient data, leading to numerous evaluations of screening uptake and determinants, and subsequential publication screening effectivenes and limitation. At the same time, there was growing emphasis in public health on disparities and social determinants. Systematic reviews, analysing screening uptake show an extensive ampunt of research in these years, particularly for breast cancer screening, reflecting its global public health importance and the need to understand low participation across diverse contexts (21).

As expected, the majority of published studies comes from high-income countries with a long tradition of well-established, population-based breast cancer screening programmes, including the United States (as far as 34%), England, the Netherlands, Denmark, Sweden, Italy, and Australia. From a geographical perspective, Europe accounts for the largest share of publications – 43.7%, reflecting a large number of countries with implementation of organized screening programmes and the evaluation of long-term programme data.

Overall, the geographic distribution of the included studies demonstrates a pronounced concentration of evidence originating from countries with well-established, organized breast cancer screening systems. In contrast, there is comparatively limited data from low- and middle-income regions, where organized screening programmes are less effectively implemented or have been introduced more recent, reflecting differences in health system infrastructure and data availability. Since the data were obtained from high-income countries, the results and potential implications cannot be

generalized to all countries, particularly developing and underdeveloped countries.

Figure 1. PRISMA flow chart



The included studies showed a range of methodology designs, with a clear predominance of quantitative methodologies (Table 2, Suplementary Table). while small number of studies (10.7%) were using mixed-methods designs that integrated both qualitative and quantitative approaches (Table 2), where comparison between subject perceptions and objectively measured screening participation can be evaluated. The predominance of quantitative analyses can be explained by the availability of data sources such as registry-based analyses and observational cohort designs, which were used retrospectively and could not be matched with participants' perceptions at the time of data processing and publication.Breast cancer screening can be performed as organized (population-based), opportunistic, or risk-adapted (personalised approaches). - tend to individualize screening based on estimated risk and at the same time retaining key elements of organized programmes, but their real world practice implementation is still limited (22, 23).

In our review, the analysis showed that nearly half of the included studies focused on organized, population-based screening programmes. In contrast, studies addressing opportunistic screening accounted for 22.3% of the included literature (Table 2). In these studies, screening participation was typically assessed using data from primary health care settings and medical services (Supplementary Table), where screening uptake was largely driven by individual motivation and physician recommendation rather than systematic invitation (24). However, almost one-fifth of all included studies compared organized and opportunistic screening approaches without clearly distinguishing between these screening contexts, often drawing conclusions based on overlapping or poorly defined definitions.

The majority of included studies examined the general population of age-eligible women, accounting for 77.7% of all analysed publications. This predominance reflects the design of most organized breast cancer screening programmes, which target women within predefined age ranges and generate large, routinely collected datasets suitable for population-level analyses.

A substantial proportion of studies (13.6%) focused on migrant populations (25-35) or ethnic minority groups (36-40). There are a consistent evidence emphasising lower screening participation in these populations, attributed to factors such as language barriers, limited health literacy, cultural beliefs, mistrust of health systems, and structural obstacles related to access. Migrants are less likely to participate in mammography screening programmes compared with local-born populations in Europe (30). Migrant women, born in countries with breast cancer low incidence are more likely to perceive higher risk of developing breast cancer with length of stay in developed county, with higher breast cancer incidence (30). Opposite to that, 87% of Asian immigrant to Canada had mammogram during their lifetime, at least once (organized or opportunistic) (25). A study, conducted by Kristiansen et al. using data from 1991-2008, showed that lack of contact with medical professionals and unemployment significantly affect efficacy of breast cancer screening, in negative way (27). Among ethnic minority group, greater perceived discrimination was associated with poorer health care–a results published by Gonzales et al, showed that among American Indian women, discrimination in health care settings may contribute to lower participation in breast and other cancer screening services (37).

Vulnerable populations remain insufficiently represented relative to their importance for screening equity. Minority based approach to breast cancer screening should take into account targeted culturally adapted strategies, rather than using general, population-wide approaches. Potential interventions that are derive from this results can be community based communication, multilingual education and investing efforts in strengthening connections and trust between these vulnerable groups and healthcare providers.

In contrast, only 2.9% of studies specifically addressed women of low socioeconomic status (41-43), despite well-documented associations between socioeconomic disadvantage and reduced screening uptake. Similarly, women residing in rural or geographically remote areas were underrepresented, with only 1.3% of studies focusing on this population, accentuating distance, transportation difficulties, and limited availability of health services as key barriers (42, 44). Fewer than 4% of the included studies examined women with disabilities or those with a previous false-positive screening result (45-50). Studies involving women with

disabilities reported barriers related to physical accessibility and inadequate communication within screening services, whereas studies addressing women with prior false-positive results emphasized psychological distress, anxiety, and reduced trust in screening, which negatively affected further screening participation.

Overall, the literature highlights social, cultural, and geographic inequalities in breast cancer screening participation, indicating that these disparities remain insufficiently addressed across different health care systems and population groups. Data are presented in Table 2 and Supplementary Table. This heterogeneity is consistent with the objectives of a scoping review and enabled comprehensive mapping of the wide range of barriers affecting breast cancer screening participation across different healthcare systems and sociocultural contexts.

The multifactorial nature of barriers to effective breast cancer screening is clearly illustrated in Table 3, where substantial overlap between different limitations can be clearly observed in Table 4. Nevertheless, for the purposes of this review and in accordance with the published literature, barrier domains were categorized into the following groups (Table 3): patient-level barriers; access-related barriers; health system and organizational barriers; socioeconomic and cultural barriers; and provider-related barriers. The key barriers identified across these domains are summarized in Table 4.

Patient-level barriers were the most frequently reported, identified in 78 studies (75.7%). These barriers primarily encompassed fear of mammography-related pain, anxiety about a potential cancer diagnosis or subsequent treatment, fear from radiation exposure, and low perceived personal risk of breast cancer, particularly among asymptomatic women (51-79).

This clear predominance of patient-centered barriers should not be perceived as proof that women themselves are primary reason for screening non-participation. Number of here-defined individual barriers show substantial connection to structural and social determinants (transport limitations, competing work, low health literacy, financial insecurity, cultural marginalization, etc.). These reasons are notably significant in underserved populations, where barriers may accumulate across several domains at the same time. These findings clearly support establishing a broader interpretative framework for the reasons behind non-participation in screening, encompassing not only personal beliefs and choices, but also factors related to the healthcare system and inequalities between the groups being studied. A study conducted by Speedy and Hase, investigated influence of health beliefs—response efficacy, perceived seriousness, concern, susceptibility, and perceived barriers—on the likelihood of attending breast cancer screening. Compared with women who had not undergone mammography, screening participants were more health-conscious, more likely to have previously undergone mammography, more aware of the protective role of mammography in reducing severe breast cancer, and less concerned about the screening procedure (68). Using theory of planned behaviour, Steadman ad Rutter, included over 1600 patient planned for screening mammography in UK for a questionnaire, showing determining important association and subjective norm measures have positive prediction for screening attendance (57). When difference between organized and opportunistic screening are considering, women using opportunistic screening perceived higher breast cancer risk but expressed limited awareness and concerns regarding organized screening, whereas non-screened women showed polarized risk perceptions, questioned screening usefulness, and held negative views toward mammography (78). Multivariate analyses indicated that defensive avoidance of breast cancer screening was associated with lower perceived susceptibility and response efficacy, higher breast cancer–related fear, and breast self-examination behavior – as reported in study conducted by Ivanova and Kvalem (55).

These findings direct system toward potential practical implication, that screening uptake improvements must extend beyond patient knowledge and basic health education and therefore adopt a multilevel approach, taking into account individual and structural determinants of non-participation. Potential measures here can include concern adapted education, organized transportation and reminder system as well as patient navigation, which can be especially effective in vulnerable populations, where barriers often cross over multiple domains simultaneously.

Patient-related barriers are deeply connected with social determinants of health - they interact synergistically, affecting screening participation. Across the reviewed literature, the most frequently reported limitations included low income, lack of health insurance or underinsurance, and indirect costs such as transportation expenses and loss of income due to time off work. These financial constraints were further accompanied often by low educational attainment, limited health literacy, and social deprivation, which collectively reduced awareness of screening potential usefulness. Notably, financial insecurity persisted as a significant barrier even in settings where breast cancer screening was provided at no direct cost (80-95).

Here, in must be noted that a majority of available evidence in high-income countries with long-established organized screening programmes. On the other side, evidence from low- and middle-income countries and/or settings with less developed screening infrastructure is quite limited or non-existing. This imbalance, as noted before, limits the generalizability of the available evidence and suggests that important barriers in resource-constrained settings may remain overlocked.

Deficits in health literacy and social deprivation, together with financial barriers, have been consistently reported across the globe, irrespective of countries' income level. These barriers persist across diverse health-care systems and screening models, suggesting that economic development alone is insufficient to ensure equitable screening

participation (80, 81, 87, 90, 95). A cross-sectional study conducted in 2017. showed that participation in breast cancer screening programs was associated with woman health insurance, while breast cancer awareness was associated with lack of social deprivation and woman occupation (85). A Flanders study showed that patient with crowded households, population densities and employment status will more likely to undergo outside organized screening system, that has more coverage rate (86). A study by Zhao et al, published in 2025. showed that Gross Domestic Product (GDP) per capita has negative influence on mammography nonattendance, while urbanization showed positive correlation (90).

Access-related barriers are encompassing geographic and service-related constraints. Key barriers included long distances to screening facilities, transportation difficulties, inconvenient appointment times, limited availability of screening services, and prolonged waiting times. These challenges were particularly pronounced in rural and remote areas, contributing to difference in urban/rural disparities (89). In addition, inadequate accessibility of screening and/or facilities is a significant barrier for women with disabilities, further accentuating inequities in screening participation (50). Access-related barriers are wide term, overlapping with health system and provider barriers (95-100).

Among previously unscreened women from disadvantaged areas, screening uptake was higher among those living closer to screening clinics (95), while women with private vehicle would not ephasise time as a barier to further mamography facility (97). In favour to acessibility, speaks a large study in Denmark, on almost 150.000 participants, stating that a long travel distance is associated with an increased risk of non-participation (100).

Literature findings emphasize that improving screening participation should prioritize accessibility and social equity, together with advances in healthcare infrastructure, such as mobile screening services, shorter waiting lists, adapted facilities, thus reducing structural disadvantages across healthcare settings.

Health system and organizational barriers reflected structural inefficiencies within screening programmes, including delayed or failed invitation processes, registry inaccuracy, and the absence of effective, well established reminder system. Additional obstacles included insufficiently defined care pathways, lack of individual approach, insufficient screening capacity causing prolonged intervals, and administrative complexity and errors, all of which collectively undermined adequate access to screening participation, as shown in included studies (101-111).

Several studies highlighted deficiencies in reminder systems—including inadequate, delayed, or absent reminders—as a key organizational barrier, emphasizing their critical role in sustaining engagement and continuity in breast cancer screening participation (101, 106, 109, 110). Confirmation requirements constitute an obstacle mainly for women invited for the first time, while having little influence on participation among those with previous screening experience (108).

Provider-related barriers primarily reflected limitations in provider (medical staff)- subject interactions and communication processes. These included the lack of sufficient physician recommendations for screening, short counseling time, and inadequate communication regarding the benefits, risks, and procedures of mammography. In addition, unclear screening guidelines, limited cultural competence, and language barriers during clinical encounters further hindered informed decision-making and reduced screening participation, particularly among socioeconomically and culturally diverse populations, especially the ones with language barriers (112-124).

Women who had undergone mammography were more likely to report receiving a recommendation for screening, having an established primary care provider, and being cared for by a female primary care provider (113). A brief, qualitative study, conducted by Sterlingova and Lunden identified non-personalization of the screening system as a dominant theme, reflecting participants' perceptions of limited individualization (115).

In a twenty-year-old study and with still actual barriers, published by Aro et al, the most common reason for non-attendance was having undergone mammography outside the organized programme. Other reported reasons included logistical barriers, fear and anxiety, limited screening knowledge, and organizational issues. Non-attenders could be broadly classified into two groups; women screened elsewhere were typically urban, socioeconomically advantaged, health-proactive, perceived themselves at higher risk of breast cancer, and expected the examination to be painful (117).

These findings clearly illustrate the complexity and interrelated nature of barriers influencing decisions to participate in screening. Simplified classification models may be insufficient to identify the true constraints of non-attendance, with important implications for the design and optimization of screening programmes.

From a practical perspective, the findings indicate that isolated interventions are unlikely to substantially improve screening uptake. More effective strategies are likely to require coordinated, multilevel approaches that combine public education, culturally and linguistically appropriate communication, improved invitation and reminder systems, easier appointment scheduling, better physical and geographic access to services, and stronger provider engagement in screening recommendation and counseling. In organized screening programmes, particular attention should be given to registry quality, invitation accuracy, recall systems, and continuity of follow-up. In underserved populations, tailored outreach strategies and more flexible service delivery models may be especially important.

A small number of studies (n = 3) did not provide sufficiently precise definitions of screening barriers; however, their research focus was considered methodologically relevant and aligned with the objectives of this review (124-127).

Although the analyzed data from the included studies provided valuable insight into breast cancer screening programs and identified key target domains in which major barriers to effective screening implementation can be recognized—potentially serving as a framework for future guidance and for the development of efficient, organized screening systems—several limitations should be acknowledged to inform further actions aimed at improving screening. First, this scoping review included studies published between 2000 and 2025; consequently, some barriers identified earlier may not have been captured. However, given the extended period evaluated, it can be reasonably assumed that such barriers have by now been clearly identified and, in many cases, appropriately addressed. Nevertheless, despite the fact that breast cancer screening remains insufficiently effective in many countries, there is a notable lack of data from the past five years—particularly studies that would identify emerging barriers to successful screening, as well as those indicating whether barriers reported in earlier studies have been successfully resolved.

In addition to that, grey literature was not systematically searched, and intervention studies were not the primary focus of this review; therefore, some potentially relevant implementation evidence may not have been captured.

The included studies were methodologically heterogeneous in terms of design, populations, screening settings, and reported outcomes, which precluded quantitative synthesis.

Finally, the predominance of evidence from high-income countries limits the transferability of the findings to low-resource settings. Despite these limitations, the scoping approach allowed broad mapping of the literature and identification of recurring domains that may inform future research and programme redesign.

CONCLUSION

This scoping review defined key domains encompassing participant- or population-level factors, access and health system organization, cultural and socioeconomic domains, as well as factors related to healthcare providers and the health system. Most of the available evidence originates from high-income regions with well-organized screening systems and high population coverage, whereas data from low- and middle-income countries, where breast cancer screening remains less effective, are scarce. Disadvantaged populations, as previously defined, have been insufficiently represented in research, despite a growing emphasis in recent years on equity-sensitive and vulnerable populations. The findings of this review may help support future screening improvement efforts by emphasizing the need for coordinated action across multiple domains, rather than isolated interventions targeting a single barrier.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Agreements No. 451-03-34/2026-03/200111).

This research has also received funding from the Faculty of Medical Sciences, University of Kragujevac, Serbia (JP04/24).

REFERENCES

1. OECD. Cancer screening: Health at a Glance 2023. OECD; 2023. Accessed January 15, 2026. https://www.oecd.org/en/publications/health-at-a-glance-2023_7a7afb35-en/full-report/cancer-screening_212c79aa.html
2. OECD. Beating Cancer Inequalities in the EU: Spotlight on Cancer Prevention and Early Detection. OECD Publishing; 2024. Accessed January 15, 2026. https://www.oecd.org/en/publications/beating-cancer-inequalities-in-the-eu_14fdc89a-en.html
3. Srinath A, van Merode F, Rao SV, Pavlova M. Barriers to cervical cancer and breast cancer screening uptake in low- and middle-income countries: a systematic review. Health Policy Plan. 2022;38(4):509–27.
4. Aliu AE, Kerrison RS, Marcu A. A Systematic Review of Barriers to Breast Cancer Screening, and of Interventions Designed to Increase Participation, Among Women of Black African and Black Caribbean Descent in the UK. Psychooncology. 2025;34(2):e70093.
5. Nduka IJ, Ejie IL, Okafor CE, Eleje GU, Ekwunife OI. Interventions to increase mammography screening uptake among women living in low-income and middle-income countries: a systematic review. BMJ Open. 2023;13(2):e066928.
6. Testing the European quality assurance scheme | Cancer Screening, Diagnosis and Care. Accessed January 15, 2026. https://cancer-screening-and-care.jrc.ec.europa.eu/en/ecibc/breast-quality-assurance-scheme/testing-the-scheme
7. Zotero [computer software]. Corporation for Digital Scholarship; 2025. Accessed January 15, 2026. https://www.zotero.org/
8. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan—a web and mobile app for systematic reviews. Syst Rev. 2016;5(1):210.
9. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(5):1-31. doi:10.3322/caac.21834.
10. Xu Y, Gong M, Wang Y. et al. Global trends and forecasts of breast cancer incidence and deaths. Sci Data 10,

334 (2023). https://doi.org/10.1038/s41597-023-02253-5

11. Kim J, Harper A, McCormack V, Sung H, Houssami N, Morgan E, et al. Global patterns and trends in breast cancer incidence and mortality across 185 countries. Nat Med. 2025;31(4):1154-1162. doi: 10.1038/s41591-025-03502-3.
12. Freihat O, Sipos D, Kovacs A. Global burden and projections of breast cancer incidence and mortality to 2050: a comprehensive analysis of GLOBOCAN data. Front Public Health. 2025 Oct 30;13:1622954. doi: 10.3389/fpubh.2025.1622954.
13. Mittmann N, Seung SJ, Ante Z, Liu N, Yong JH, Yusuf A, et al. Updated breast cancer costs for women by disease stage and phase of care using population-based databases. Health Rep. 2024;35(11):3-11. doi: 10.25318/82-003-x202401100001-eng.
14. Henderson JT, Webber EM, Weyrich MS, Miller M, Melnikow J. Screening for Breast Cancer: Evidence Report and Systematic Review for the US Preventive Services Task Force. JAMA. 2024 Jun 11;331(22):1931-1946. doi: 10.1001/jama.2023.25844. PMID: 38687490.
15. Oeffinger KC, Fontham ET, Etzioni R, Herzig A, Michaelson JS, Shih YC, et al. American Cancer Society. Breast Cancer Screening for Women at Average Risk: 2015 Guideline Update From the American Cancer Society. JAMA. 2015;314(15):1599-614. doi: 10.1001/jama.2015.12783. Erratum in: JAMA. 2016;315(13):1406. doi: 10.1001/jama.2016.3404.
16. Katsika L, Boureka E, Kalogiannidis I, Tsakiridis I, Tirodimos I, Lallas K, et al. Screening for Breast Cancer: A Comparative Review of Guidelines. Life (Basel). 2024;14(6):777. doi: 10.3390/life14060777.
17. Pleasant V, Bapaye C, Delporte F, Vigil MJDR, Divakar H, Delgado GF, et al. FIGO Committee on Breast Health. National policies for screening and early detection of breast cancer around the globe: Practices, barriers, and solutions. Int J Gynaecol Obstet. 2025;171(3):1046-1053. doi: 10.1002/ijgo.70545.
18. Cardoso R, Hoffmeister M, Brenner H. Breast cancer screening programmes and self-reported mammography use in European countries. Int J Cancer. 2023;152(12):2512-2527. doi: 10.1002/ijc.34494.
19. Bayrakçeken E, Yaralı S, Alkan Ö. Identify risk factors affecting participation of Turkish women in mammography screening for breast cancer prevention. Breast Cancer Res Treat. 2024;205(3):487-495. doi: 10.1007/s10549-024-07296-9.
20. Tavakoli B, Feizi A, Zamani-Alavijeh F, Shahnazi H. Factors influencing breast cancer screening practices among women worldwide: a systematic review of observational and qualitative studies. BMC Womens Health. 2024;24(1):268. doi: 10.1186/s12905-024-03096-x.
21. Aguiar-Ibáñez R, Mbous Y, Sharma S, Chakali R, Chawla E. Barriers to cancer screening uptake and approaches to overcome them: a systematic literature review. Front Oncol. 2025;15:1575820. doi: 10.3389/fonc.2025.1575820.
22. Luo C, Wang L, Zhang Y, Lu M, Lu B, Cai J, et al. Advances in breast cancer screening modalities and status of global screening programs. Chronic Dis Transl Med. 2022;8(2):112-123. doi: 10.1002/cdt3.21.
23. Schünemann HJ, Lerda D, Quinn C, Follmann M, Alonso-Coello P, Rossi PG, et al; European Commission Initiative on Breast Cancer (ECIBC) Contributor Group. Breast Cancer Screening and Diagnosis: A Synopsis of the European Breast Guidelines. Ann Intern Med. 2020;172(1):46-56. doi: 10.7326/M19-2125.
24. Li H, Huang M, Yang Y, Tang J, Ye Y. The Practice and Willingness of Women Towards Opportunistic Screening for Breast and Cervical Cancers in Sichuan Province, China: A Cross-Sectional Study. Risk Manag Healthc Policy. 2023;16:169-183. doi: 10.2147/RMHP.S391534.
25. Hippman C, Moshrefzadeh A, Lohn Z, Hodgson ZG, Dewar K, Lam M, et al. Breast Cancer and Mammography Screening: Knowledge, Beliefs and Predictors for Asian Immigrant Women Attending a Specialized Clinic in British Columbia, Canada. J Immigr Minor Health. 2016;18(6):1441-1448. doi: 10.1007/s10903-015-0332-8. PMID: 26706472.
26. Lue Kessing L, Norredam M, Kvernrod AB, Mygind A, Kristiansen M. Contextualising migrants' health behaviour - a qualitative study of transnational ties and their implications for participation in mammography screening. BMC Public Health. 2013;13:431. doi: 10.1186/1471-2458-13
27. Kristiansen M, Thorsted BL, Krasnik A, von Euler-Chelpin M. Participation in mammography screening among migrants and non-migrants in Denmark. Acta Oncol. 2012 Jan;51(1):28-36. doi: 10.3109/0284186X.2011.626447. Epub 2011 Oct 30. PMID: 22035117.
28. Kobetz E, Menard J, Barton B, Maldonado JC, Diem J, Auguste PD, et al. Barriers to breast cancer screening among Haitian immigrant women in Little Haiti, Miami. J Immigr Minor Health. 2010;12(4):520-6. doi: 10.1007/s10903-010-9316-x. PMID: 20091231.
29. Suwankhong D, Liamputtong P. Early Detection of Breast Cancer and Barrier to Screening Programmes amongst Thai Migrant Women in Australia: A Qualitative Study. Asian Pac J Cancer Prev. 2018;19(4):1089-1097. doi: 10.22034/APJCP.2018.19.4.1089. PMID: 29699369; PMCID: PMC6031773.
30. Kristiansen M, Lue-Kessing L, Mygind A, Razum O, Norredam M. Migration from low- to high-risk countries: a qualitative study of perceived risk of breast cancer and the influence on participation in mammography screening among migrant women in Denmark. Eur J Cancer Care (Engl). 2014;23(2):206-13. doi: 10.1111/ecc.12100. Epub 2013 Jul 16. PMID: 23855488.
31. Berens EM, Yilmaz-Aslan Y, Spallek J, Razum O. Determinants of mammography screening participation among Turkish immigrant women in Germany--a qualitative study reflecting key informants' and women's perspectives. Eur J Cancer Care (Engl). 2016;25(1):38-48. doi: 10.1111/ecc.12334. Epub 2015 Jun 5. PMID: 26052964.

32. Fayanju OM, Kraenzle S, Drake BF, Oka M, Goodman MS. Perceived barriers to mammography among underserved women in a Breast Health Center Outreach Program. Am J Surg. 2014;208(3):425-34. doi: 10.1016/j.amjsurg.2014.03.005. Epub 2014 May 4. PMID: 24908357; PMCID: PMC4135000.
33. Ahmad F, Mahmood S, Pietkiewicz I, McDonald L, Ginsburg O. Concept mapping with South Asian immigrant women: barriers to mammography and solutions. J Immigr Minor Health. 2012;14(2):242-50. doi: 10.1007/s10903-011-9472-7. PMID: 21538023.
34. Jacobs EA, Karavolos K, Rathouz PJ, Ferris TG, Powell LH. Limited English proficiency and breast and cervical cancer screening in a multiethnic population. Am J Public Health. 2005 Aug;95(8):1410-6. doi: 10.2105/AJPH.2004.041418. PMID: 16043670; PMCID: PMC1449374.
35. Garbers S, Jessop DJ, Foti H, Uribelarrea M, Chiasson MA. Barriers to breast cancer screening for low-income Mexican and Dominican women in New York City. J Urban Health. 2003 Mar;80(1):81-91. doi: 10.1007/pl00022327. PMID: 12612098; PMCID: PMC3456108.
36. Andrew A, Kima J, Rahul C, Letendre A, James A, Chiang B, et al. Barriers to and facilitators of breast, cervical and colorectal cancer screening and cancer screening histories of Métis people in Alberta, Canada. Int J Circumpolar Health. 2024;83(1):2368766. doi: 10.1080/22423982.2024.2368766. Epub 2024 Jun 19. PMID: 39612358; PMCID: PMC11188950.
37. Gonzales KL, Harding AK, Lambert WE, Fu R, Henderson WG. Perceived experiences of discrimination in health care: a barrier for cancer screening among American Indian women with type 2 diabetes. Womens Health Issues. 2013;23(1):e61-7. doi: 10.1016/j.whi.2012.10.004. PMID: 23312714; PMCID: PMC3640290.
38. Watson-Johnson LC, DeGroff A, Steele CB, Revels M, Smith JL, Justen E, et al. Mammography adherence: a qualitative study. J Womens Health (Larchmt). 2011;20(12):1887-94. doi: 10.1089/jwh.2010.2724. Epub 2011 Oct 24. PMID: 22023414.
39. Thomas VN, Saleem T, Abraham R. Barriers to effective uptake of cancer screening among Black and minority ethnic groups. Int J Palliat Nurs. 2005;11(11):562, 564-71. doi: 10.12968/ijpn.2005.11.11.20096. PMID: 16471043.
40. Kwok C, Fethney J, White K. Mammographic screening practices among Chinese-Australian women. J Nurs Scholarsh. 2012;44(1):11-8. doi: 10.1111/j.1547-5069.2011.01429.x. Epub 2011 Dec 12. PMID: 22151348.
41. Schwartz K, Fakhouri M, Bartoces M, Monsur J, Younis A. Mammography screening among Arab American women in metropolitan Detroit. J Immigr Minor Health. 2008;10(6):541-9. doi: 10.1007/s10903-008-9140-8. PMID: 18392934.
42. Seetoh T, Siew WF, Koh A, Liau WF, Koh GC, Lee JJ, et al. Overcoming Barriers to Mammography Screening: A Quasi-randomised Pragmatic Trial in a Community-based Primary Care Setting. Ann Acad Med Singap. 2014;43(12):588-94. PMID: 25588917.
43. Garza MA, Luan J, Blinka M, Farabee-Lewis RI, Neuhaus CE, Zabora JR, et al. A culturally targeted intervention to promote breast cancer screening among low-income women in East Baltimore, Maryland. Cancer Control. 2005;12 Suppl 2:34-41. doi: 10.1177/1073274805012004S06. PMID: 16327749.
44. Mayo RM, Ureda JR, Parker VG. Importance of fatalism in understanding mammography screening in rural elderly women. J Women Aging. 2001;13(1):57-72. doi: 10.1300/J074v13n01_05. PMID: 11217186.
45. Drossaert, C. H. C., Boer, H., & Seydel, E. R.. Prospective Study on the Determinants of Repeat Attendance and Attendance Patterns in Breast Cancer Screening Using the Theory of Planned Behaviour. Psychology & Health 2003;18(5), 551–565. https://doi.org/10.1080/08870440310001412 07
46. Truesdale-Kennedy M, Taggart L, McIlfatrick S. Breast cancer knowledge among women with intellectual disabilities and their experiences of receiving breast mammography. J Adv Nurs. 2011;67(6):1294-304. doi: 10.1111/j.1365-2648.2010.05595.x. Epub 2011 Mar 1. PMID: 21366669.
47. Hofvind SS, Wang H, Thoresen S. The Norwegian Breast Cancer Screening Program: re-attendance related to the women's experiences, intentions and previous screening result. Cancer Causes Control. 2003;14(4):391-8. doi: 10.1023/a:1023918610664. PMID:12846372.
48. Akwo JD, Erim AE, Ikamaise VC, Archibong B, Ekpo EU. Transforming Screening Uptake in Low-resource and Underinformed Populations: A Preliminary Study of Factors Influencing Women's Decisions to Uptake Screening. J Med Imaging Radiat Sci. 2019;50(2):323-330.e2. doi: 10.1016/j.jmir.2019.02.003. Epub 2019 Mar 23. PMID: 31176441.
49. Jarman MP, Bowling JM, Dickens P, Luken K, Yankaskas BC. Factors facilitating acceptable mammography services for women with disabilities. Womens Health Issues. 2012;22(5):e421-8. doi: 10.1016/j.whi.2012.06.002. Epub 2012 Jul 17. PMID: 22818248; PMCID: PMC3433570.
50. Yankaskas BC, Dickens P, Bowling JM, Jarman MP, Luken K, Salisbury K, et al. Barriers to adherence to screening mammography among women with disabilities. Am J Public Health. 2010;100(5):947-53. doi: 10.2105/AJPH.2008.150318. Epub 2009 Oct 15. PMID: 19834002; PMCID: PMC2853618.
51. Pivot X, Rixe O, Morere J, Coscas Y, Cals L, Namer M, et al. Breast cancer screening in France: results of the EDIFICE survey. Int J Med Sci. 2008;5(3):106-12. doi: 10.7150/ijms.5.106. PMID: 18566655; PMCID: PMC2407526.
52. Alamo-Junquera D, Murta-Nascimento C, Macià F, Baré M, Galcerán J, Ascunce N, et al. Cumulative False-Positive Risk Group. Effect of false-positive results on reattendance at breast cancer screening programmes in Spain. Eur J Public Health. 2012;22(3):404-8. doi:

10.1093/eurpub/ckr057. Epub 2011 May 9. PMID: 21558152.
53. Woof VG, Ruane H, Ulph F, French DP, Qureshi N, Khan N, et al. Engagement barriers and service inequities in the NHS Breast Screening Programme: Views from British-Pakistani women. J Med Screen. 2020;27(3):130-137. doi: 10.1177/0969141319887405. Epub 2019 Dec 2. PMID: 31791172; PMCID: PMC7645618.
54. Sullivan SG, Slack-Smith LM, Hussain R. Understanding the use of breast cancer screening services by women with intellectual disabilities. Soz Praventivmed. 2004;49(6):398-405. doi: 10.1007/s00038-004-3121-z. PMID: 15669440.
55. Ivanova A, Kvalem IL. Psychological predictors of intention and avoidance of attending organized mammography screening in Norway: applying the Extended Parallel Process Model. BMC Womens Health. 2021;21(1):67. doi: 10.1186/s12905-021-0F1201-y. PMID: 33588809; PMCID: PMC7885347.
56. Jafari M, Nakhaee N, Goudarzi R, Zehtab N, Barouni M. Participation of the Women Covered by Family Physicians in Breast Cancer Screening Program in Kerman, Iran. Asian Pac J Cancer Prev. 2015;16(11):4555-61. doi: 10.7314/apjcp.2015.16.11.4555. PMID: 26107203.
57. Steadman L, Rutter DR. Belief importance and the theory of planned behaviour: comparing modal and ranked modal beliefs in predicting attendance at breast screening. Br J Health Psychol. 2004;9(Pt 4):447-63. doi: 10.1348/1359107042304579. PMID: 15509354.
58. Dundar PE, Ozyurt BC, Erdurak K. Sociodemographic determinants of nonattendance in a population-based mammography screening program in the city of Manisa, Turkey. ScientificWorldJournal. 2012;2012:816903. doi: 10.1100/2012/816903. Epub 2012 Mar 12. PMID: 22489204; PMCID: PMC3317549.
59. Marmarà D, Marmarà V, Hubbard G. Predicting reattendance to the second round of the Maltese national breast screening programme: an analytical descriptive study. BMC Public Health. 2019;19(1):189. doi: 10.1186/s12889-019-6507-9. PMID: 30760275; PMCID: PMC6374893.
60. Rauscher GH, Hawley ST, Earp JA. Baseline predictors of initiation vs. maintenance of regular mammography use among rural women. Prev Med. 2005;40(6):822-30. doi: 10.1016/j.ypmed.2004.09.029. PMID: 15850884.
61. Allahverdipour H, Asghari-Jafarabadi M, Emami A. Breast cancer risk perception, benefits of and barriers to mammography adherence among a group of Iranian women. Women Health. 2011;51(3):204-19. doi: 10.1080/03630242.2011.564273. PMID: 21547858.
62. Esteva M, Ripoll J, Leiva A, Sánchez-Contador C, Collado F. Determinants of non attendance to mammography program in a region with high voluntary health insurance coverage. BMC Public Health. 2008;8:387. doi: 10.1186/1471-2458-8-387. PMID: 19014522; PMCID: PMC2596126.
63. Hassan N, Ho WK, Mariapun S, Teo SH. A cross sectional study on the motivators for Asian women to attend opportunistic mammography screening in a private hospital in Malaysia: the MyMammo study. BMC Public Health. 2015;15:548. doi: 10.1186/s12889-015-1892-1. PMID: 26065413; PMCID: PMC4465302.
64. Bener A, Honein G, Carter AO, Da'ar Z, Miller C, Dunn EV. The determinants of breast cancer screening behavior: a focus group study of women in the United Arab Emirates. Oncol Nurs Forum. 2002;29(9):E91-8. doi: 10.1188/02.ONF.E91-E98. PMID: 12370705.
65. Al-Mousa DS, Spuur K, Attar R, Kleib I, Alakhras M. Knowledge, attitudes, and practices related to breast cancer screening among female Jordanian university employees: A cross-sectional study. Radiography (Lond). 2024;30(1):258-264. doi: 10.1016/j.radi.2023.11.004. Epub 2023 Nov 29. PMID: 38035443.
66. Carney PA, Harwood BG, Greene MA, Goodrich ME. Impact of a telephone counseling intervention on transitions in stage of change and adherence to interval mammography screening (United States). Cancer Causes Control. 2005;16(7):799-807. doi: 10.1007/s10552-005-2612-4. PMID: 16132790.
67. Hamshari S, Nazzal Z, Altell M, Nanaa I, Jbara R, Sabri R. Mammogram uptake and barriers among Palestinian women attending primary health care in North Palestine. Eur J Gen Pract. 2021;27(1):264-270. doi: 10.1080/13814788.2021.1985996. PMID: 34633262; PMCID: PMC8510586.
68. Speedy S, Hase S. Health beliefs and perceptions of women presenting or not presenting for mammographic screening in a rural health setting. Aust J Rural Health. 2000;8(4):208-13. doi: 10.1046/j.1440-1584.2000.00273.x. PMID: 11894286.
69. Lagerlund M, Widmark C, Lambe M, Tishelman C. Rationales for attending or not attending mammography screening--a focus group study among women in Sweden. Eur J Cancer Prev. 2001;10(5):429-42. doi: 10.1097/00008469-200110000-00007. PMID: 11711758.
70. McBride KA, Fleming CAK, George ES, Steiner GZ, MacMillan F. Double Discourse: Qualitative Perspectives on Breast Screening Participation among Obese Women and Their Health Care Providers. Int J Environ Res Public Health. 2019;16(4):534. doi: 10.3390/ijerph16040534. PMID: 30781792; PMCID: PMC6407106.
71. Friedman AM, Hemler JR, Rossetti E, Clemow LP, Ferrante JM. Obese women's barriers to mammography and pap smear: the possible role of personality. Obesity (Silver Spring). 2012;20(8):1611-7. doi: 10.1038/oby.2012.50.Epub 2012 Feb 28. PMID: 22370590; PMCID: PMC3378788.
72. Davis TC, Arnold CL, Rademaker A, Bailey SC, Platt DJ, Reynolds C, Esparza J, Liu D, Wolf MS. Differences in barriers to mammography between rural and urban women. J Womens Health (Larchmt). 2012;21(7):748-55. doi: 10.1089/jwh.2011.3397. Epub 2012 Apr 20. PMID: 22519704; PMCID: PMC3432575.
73. Farmer D, Reddick B, D'Agostino R, Jackson SA. Psychosocial correlates of mammography screening in older African American women. Oncol Nurs Forum.

2007;34(1):117-23. doi: 10.1188/07.ONF.117-123. PMID: 17562638.
74. Pfeffer N. Screening for breast cancer: candidacy and compliance. Soc Sci Med. 2004;58(1):151-60. doi: 10.1016/s0277-9536(03)00156-4. PMID: 14572928.
75. Al-Zalabani AH, Alharbi KD, Fallatah NI, Alqabshawi RI, Al-Zalabani AA, Alghamdi SM. Breast Cancer Knowledge and Screening Practice and Barriers Among Women in Madinah, Saudi Arabia. J Cancer Educ. 2018;33(1):201-207. doi: 10.1007/s13187-016-1057-7. PMID: 27271153.
76. Lostao L, Joiner TE, Pettit JW, Chorot P, Sandín B. Health beliefs and illness attitudes as predictors of breast cancer screening attendance. Eur J Public Health. 2001;11(3):274-279. doi:10.1093/eurpub/11.3.274
77. Ferrat E, Le Breton J, Djassibel M, Veerabudun K, Brixi Z, Attali C, et al. Understanding barriers to organized breast cancer screening in France: women's perceptions, attitudes, and knowledge. Fam Pract. 2013;30(4):445-51. doi: 10.1093/fampra/cmt004. Epub 2013 Mar 11. PMID: 23478254.
78. Adefemi K, Knight JC, Zhu Y, Wang PP. Concurrent cancer screening participation and associated factors among Canadian women: Insights from a cross-sectional study. J Med Screen. 2025;32(4):205-214. doi: 10.1177/09691413251333223. Epub 2025 Apr 21. PMID: 40259573; PMCID: PMC12569105.
79. Újhelyi M, Pukancsik D, Kelemen P, Kovács E, Kenessey I, Bak M, et al. Barriers to Organized Mammography Screening Programs in Hungary: A Questionnaire-based Study of 3,313 Women. Anticancer Res. 2018;38(3):1727-1734. doi: 10.21873/anticanres.12408. PMID: 29491109.
80. Chongthawonsatid S. Inequity of healthcare utilization on mammography examination and Pap smear screening in Thailand: Analysis of a population-based household survey. PLoS One. 2017;12(3):e0173656. doi: 10.1371/journal.pone.0173656. PMID: 28282430; PMCID: PMC5345859.
81. Mack KP, Pavao J, Tabnak F, Knutson K, Kimerling R. Adherence to recent screening mammography among Latinas: findings from the California Women's Health Survey. J Womens Health (Larchmt). 2009;18(3):347-54. doi: 10.1089/jwh.2008.0793. PMID: 19281318.
82. Wall KM, Núñez-Rocha GM, Salinas-Martínez AM, Sánchez-Peña SR. Determinants of the use of breast cancer screening among women workers in urban Mexico. Prev Chronic Dis. 2008;5(2):A50. Epub 2008 Mar 15. PMID: 18341785; PMCID: PMC2396987.
83. Bowie JV, Curbow B, Laveist TA, Fitzgerald S, and Zabora J. The Theory of Planned Behavior and Intention to Repeat Mammography Among African-American Women. Journal of Psychosocial Oncology, 2003;21(4),23–42. https://doi.org/10.1300/J077v21n04_02
84. Chouliara Z, Power KG, Swanson V, and Johnstone F. Factors associated with breast screening attendance: A controlled comparison between attenders and non-attenders in Scotland. International Journal of Health Promotion and Education, 2002;40(3), 78–90. https://doi.org/10.1080/14635240.2002.10806203
85. Ghanbari A, Rahmatpour P, Hosseini N, Khalili M. Social Determinants of Breast Cancer Screening among Married Women: A Cross-Sectional Study. J Res Health Sci. 2020;20(1):e00467. doi: 10.34172/jrhs.2020.02. PMID: 32814688; PMCID: PMC7585759.
86. Ding L, Jidkova S, Greuter MJW, Van Herck K, Goossens M, Martens P, et al. Coverage determinants of breast cancer screening in Flanders: an evaluation of the past decade. Int J Equity Health. 2020;19(1):212. doi: 10.1186/s12939-020-01323-z. PMID: 33246477; PMCID: PMC7694412.
87. Jensen LF, Pedersen AF, Andersen B, Vedsted P. Identifying specific non-attending groups in breast cancer screening - population-based registry study of participation and socio-demography. BMC Cancer 12, 518 (2012). https://doi.org/10.1186/1471-2407-12-518.
88. Lagerlund M, Hedin A, Sparén P, Thurfjell E, Lambe M. Attitudes, beliefs, and knowledge as predictors of nonattendance in a Swedish population-based mammography screening program. Prev Med. 2000;31(4):417-28. doi: 10.1006/pmed.2000.0723. PMID: 11006068.
89. Stamenić V, Strnad M. Urban-rural differences in a population-based breast cancer screening program in Croatia. Croat Med J. 2011;52(1):76-86. doi: 10.3325/cmj.2011.52.76. PMID: 21328724; PMCID: PMC3046496.
90. Zhao Q, Huang X, Ye Z, Ruan G, Wang Y, Sun P, et al. Mammography attendance in China's national breast cancer screening program: a county-level analysis of the Fujian province. BMC Womens Health. 2025;25(1):556. doi: 10.1186/s12905-025-04074-7. PMID: 41239387; PMCID: PMC12619293.
91. Unim B, Boggi R, Napoli M, Fulgenzi R, Landi A, La Torre G. Women's satisfaction with mammography and predictors of participation in an organized breast cancer screening program: Perspectives of a Local Health Unit in Rome. Public Health. 2018;155:91-94. doi: 10.1016/j.puhe.2017.11.025. Epub 2018 Jan 11. PMID: 29331770.
92. Lim SK, Teo XL, Ng JL, Li FX, Tan SM. A Survey on Singaporean Women's Knowledge, Perception and Practices of Mammogram Screening. Ann Acad Med Singap. 2015;44(9):317-25. PMID: 26584660.
93. Freitas C, Tura LF, Costa N, Duarte J. A population-based breast cancer screening programme: conducting a comprehensive survey to explore adherence determinants. Eur J Cancer Care (Engl). 2012;21(3):349-59. doi: 10.1111/j.1365-2354.2011.01305.x. Epub 2011 Nov 13. PMID: 22077789.
94. Gulten G, Memnun S, Ayse K, Aygul A, Gulcin A. Breast, cervical, and colorectal cancer screening status of a group of Turkish women. Asian Pac J Cancer Prev. 2012;13(9):4273-9. doi: 10.7314/apjcp.2012.13.9.4273. PMID: 23167327.
95. Hyndman JC, Holman CD, Dawes VP. Effect of distance and social disadvantage on the response to invitations to attend mammography screening. J Med Screen.

2000;7(3):141-5. doi: 10.1136/jms.7.3.141. PMID: 11126163.

96. Pape R, Spuur KM, Umo P. Factors contributing to low participation in mammography screening in Papua New Guinea. Radiography. 2016;22(3):e151-e158. doi:10.1016/j.radi.2016.02.002
97. Peipins LA, Graham S, Young R, Lewis B, Foster S, Flanagan B, et al. Time and distance barriers to mammography facilities in the Atlanta metropolitan area. J Community Health. 2011;36(4):675-83. doi: 10.1007/s10900-011-9359-5. PMID: 21267639; PMCID: PMC5836475.
98. Marmarà D, Marmarà V, Hubbard G. Health beliefs, illness perceptions and determinants of breast screening uptake in Malta: a cross-sectional survey. BMC Public Health. 2017;17(1):416. doi: 10.1186/s12889-017-4324-6. PMID: 28482828; PMCID: PMC5422914.
99. Lagerlund M, Maxwell AE, Bastani R, Thurfjell E, Ekbom A and Lambe M. Sociodemographic predictors of non-attendance at invitational mammography screening – a population-based register study (Sweden). Cancer Causes Control 2002;13, 73–82. https://doi.org/10.1023/A:1013978421073.
100. Jensen LF, Pedersen AF, Andersen B, Fenger-Grøn M, Vedsted P. Distance to screening site and non-participation in screening for breast cancer: a population-based study. J Public Health (Oxf). 2014;36(2):292-9. doi: 10.1093/pubmed/fdt068. Epub 2013 Jul 23. PMID: 23885026.
101. Pelullo CP, Cantore F, Lisciotto A, Di Giuseppe G, Pavia M. Organized Breast and Cervical Cancer Screening: Attendance and Determinants in Southern Italy. Cancers. 2021; 13(7):1578. https://doi.org/10.3390/cancers13071578.
102. Eichholzer M, Richard A, Rohrmann S, Schmid SM, Leo C, Huang DJ, et al. Breast cancer screening attendance in two Swiss regions dominated by opportunistic or organized screening. BMC Health Serv Res. 2016;16(1):519. doi: 10.1186/s12913-016-1760-4. PMID: 27663642; PMCID: PMC5035496.
103. Poon PKM, Tam KW, Lam T, Luk AKC, Chu WCW, Cheung P, et al. Poor health literacy associated with stronger perceived barriers to breast cancer screening and overestimated breast cancer risk. Front Oncol. 2023;12:1053698. doi: 10.3389/fonc.2022.1053698. PMID: 36686831; PMCID: PMC9850080.
104. Gierisch JM, O'Neill SC, Rimer BK, DeFrank JT, Bowling JM, Skinner CS. Factors associated with annual-interval mammography for women in their 40s. Cancer Epidemiol. 2009;33(1):72-8. doi: 10.1016/j.cdp.2009.03.001. Epub 2009 May 29. PMID: 19481879; PMCID: PMC2727566.
105. Lian M, Jeffe DB, Schootman M. Racial and geographic differences in mammography screening in St. Louis City: a multilevel study. J Urban Health. 2008;85(5):677-92. doi: 10.1007/s11524-008-9301-z. Epub 2008 Jul 12. PMID: 18622709; PMCID: PMC2527433.
106. Henriksen MJ, Guassora AD, Brodersen J. Preconceptions influence women's perceptions of information on breast cancer screening: a qualitative study. BMC Res Notes. 2015;8:404. doi: 10.1186/s13104-015-1327-1. PMID: 26336075; PMCID: PMC4557860.
107. Unim B, Boggi R, Napoli M, Fulgenzi R, Landi A, La Torre G. Predictors of Mammography Uptake Among Italian Women Aged 50-69: a Cross-sectional Study. J Cancer Educ. 2020;35(6):1089-1093. doi: 10.1007/s13187-019-01560-z. PMID: 31183766.
108. Goossens M, Tran TN. The effect on attendance of the requirement to confirm a pre-scheduled appointment in a population-based mammography screening programme. Eur J Public Health. 2025;35(2):353-358. doi: 10.1093/eurpub/ckaf028. PMID: 40037772; PMCID: PMC11967882.
109. Valent F, Sammartano F, Degano S, Dellach C, Franzo A, Gerin D, et al. Reasons for non-participation in public oncological screening programs in the Italian region Friuli Venezia Giulia. Public Health. 2020;181:80-85. doi: 10.1016/j.puhe.2019.12.005. Epub 2020 Jan 17. PMID: 31958673.
110. Kovačević J, Musil V, Sović S, Zombori D, Jureša V. How to reach 70% response rate in the national breast cancer screening program in Croatia? Acta Clin Croat. 2025;64(1):81-92. doi: 10.20471/acc.2025.64.01.09. PMID: 41340791; PMCID: PMC12671709.
111. Goossens M, Van Hal G, Van der Burg M, Kellen E, Van Herck K, De Grève J, et al. Quantifying independent risk factors for failing to rescreen in a breast cancer screening program in Flanders, Belgium. Prev Med. 2014;69:280-6. doi: 10.1016/j.ypmed.2014.10.019. Epub 2014 Oct 20. PMID: 25456812.
112. Ma GX, Gao W, Lee S, Wang M, Tan Y, Shive SE. Health seeking behavioral analysis associated with breast cancer screening among Asian American women. Int J Womens Health. 2012;4:235-43. doi: 10.2147/IJWH.S30738. Epub 2012 May 25. PMID: 22723730; PMCID: PMC3379860.
113. Méndez JE, Evans M, Stone MD. Promoters and barriers to mammography screening in multiethnic inner city patients. Am J Surg. 2009;198(4):526-8. doi: 10.1016/j.amjsurg.2009.07.002. PMID: 19800461.
114. Nekhlyudov L, Ross-Degnan D, Fletcher SW. Beliefs and expectations of women under 50 years old regarding screening mammography: a qualitative study. J Gen Intern Med. 2003;18(3):182-9. doi: 10.1046/j.1525-1497.2003.20112.x. PMID: 12648249; PMCID: PMC1494837.
115. Sterlingova T, Lundén M. Why do women refrain from mammography screening? Radiography (Lond). 2018;24(1):e19-e24. doi: 10.1016/j.radi.2017.07.006. Epub 2017 Aug 12. PMID: 29306388.
116. Keane E, Moore N, Leamy B, Scally A, McEntee MF. Identifying barriers to Irish traveller women attending breast screening. Radiography (Lond). 2022;28(2):348-352. doi: 10.1016/j.radi.2021.11.010. Epub 2021 Dec 13. PMID: 34916128.
117. Aro AR, de Koning HJ, Absetz P, Schreck M. Two distinct groups of non-attenders in an organized mammography screening program. Breast Cancer Res Treat.

2001;70(2):145-53. doi: 10.1023/a:1012939228916. PMID:11768605.
118. Hutton G, Li SJ, Quaife SL, Brentnall A, Cookson J, Jenkins J, et al. Understanding barriers to breast screening: an online survey of non-attenders as part of a service evaluation in the breast screening programme in England. BMC Public Health. 2025;25(1):2509. doi: 10.1186/s12889-025-23691-3. PMID: 40684139; PMCID: PMC12275263.
119. Kwok C, Cant R, Sullivan G. Factors associated with mammographic decisions of Chinese-Australian women. Health Educ Res. 2005;20(6):739-47. doi: 10.1093/her/cyh034. Epub 2005 May 11. PMID: 15888474.
120. Ouanhnon L, Rougé Bugat ME, Lamy S, Druel V, Delpierre C, Grosclaude P. Social and territorial inequalities in breast and cervical cancers screening uptake: a cross-sectional study in France. BMJ Open. 2022;12(2):e055363. doi: 10.1136/bmjopen-2021-055363.PMID: 35193917; PMCID: PMC8867371.
121. Partin MR, Slater JS. Promoting Repeat Mammography Use: Insights From a Systematic Needs Assessment. Health Education & Behavior. 2003;30(1):97-112. doi:10.1177/1090198102239261.
122. Jolidon V. Gender inequality and mammography screening: Does living with a partner improve women's mammography uptake? Soc Sci Med. 2022;298:114875. doi: 10.1016/j.socscimed.2022.114875. Epub 2022 Feb 28. PMID: 35276623.
123. Hardy RE, Ahmed NU, Hargreaves MK, Semenya KA, Wu L, Belay Y, et al. Difficulty in reaching low-income women for screening mammography. J Health Care Poor Underserved. 2000;11(1):45-57. doi: 10.1353/hpu.2010.0614. PMID: 10778042.
124. Brunton MA. The role of effective communication to enhance participation in screening mammography: a New Zealand case. Int J Environ Res Public Health. 2009;6(2):844-61. doi: 10.3390/ijerph6020844. Epub 2009 Feb 24. PMID: 19440417; PMCID: PMC2672349.
125. Marmarà D, Marmarà V, Hubbard G. Lifetime utilization of mammography among Maltese women: a cross-sectional survey. BMC Public Health. 2018;18(1):182. doi: 10.1186/s12889-018-5093-6. PMID: 29370835; PMCID: PMC5785821.
126. Ross E, Maguire A, Donnelly M, Mairs A, Hall C, O'Reilly D. Does poor mental health explain socio-demographic gradients in breast cancer screening uptake? A population-based study. Eur J Public Health. 2020;30(3):396-401. doi: 10.1093/eurpub/ckz220. PMID :31834366.
127. Alfailakawi O, Alostath H, Aldubian A, Alsaleh AG, Alsughayer G, Alshamran A, et al. Barriers and limitations for undergoing mammography screenings among Kuwaiti women (aged 40-69) attending primary health care centers. BMC Prim Care. 2025;26(1):295. doi: 10.1186/s12875-025-02971-2. PMID: 41023619; PMC ID:PMC12482398.

ANALYSIS OF RISK FACTORS FOR THE DEVELOPMENT OF FEMOROPOPLITEAL BYPASS GRAFT OCCLUSIONS

Nikola Mirkovic[1,2], Srdjan Stefanovic[3], Snezana Sretenovic[4], Slobodan Jankovic[3,5]

[1]*University Clinical Center of Kragujevac, Department of Vascular Surgery, Kragujevac, Serbia*
[2]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Surgery*
[3]*University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Pharmacology and toxicology*
[4]*University Clinical Center of Kragujevac, Clinic of Hematology, Kragujevac, Serbia*
[5]*University Clinical Center of Kragujevac, Department of Clinical Pharmacology, Kragujevac, Serbia*

Received: 27.05.2021.
Accepted: 27.05.2021.

Corresponding author:

Dr Nikola Mirkovic

University Clinical Center of Kragujevac, Department of Vascular Surgery, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovica 69, 34000 Kragujevac, Serbia

E-mail: drnikolamirkovic@gmail.com

UDK:

Eabr 2026; 27(2):125-133
DOI:10.2478/sjecr-2021-0085

ABSTRACT

To identify significant risk factors for femoropopliteal bypass graft occlusions.A retrospective case-control study included patients who had undergone femoropopliteal bypass due to lower extremity arterial disease at the Clinical Centre Kragujevac, Serbia, between 2008. and 2011. The cases (n = 35) were patients with femoropopliteal bypass graft occlusion which required surgical intervention. The control group (n = 70) consisted of patients without graft occlusion. The cases and controls were age and sex matched. Significant impact on graft occlusion was found for the form of femoropopliteal bypass($OR_{adjusted}$ 115.34; 95%CI 4.02,3306.55;), type of graft($OR_{adjusted}$ 81.62; 95%CI 1.03,6486.32), cardiovascular disease($OR_{adjusted}$ 55.64; 95%CI 2.63,1176.94), previous vascular procedures($OR_{adjusted}$ 51.61; 95%CI 1.10,2425.14), preoperative disease stage according to Rutherford($OR_{adjusted}$ 22.21; 95%CI 2.62,188.39), and preoperative hematocrit levels ($OR_{adjusted}$ 0.34; 95%CI 0.13,0.89). A significant synergistic effect on graft occlusion was observed for the combination of form of bypass and type of graft, combination of type of graft and previous vascular procedures, combination of form of bypass and cardiovascular diseases, combination of type of graft and cardiovascular diseases, combination of type of graft and preoperative disease stage, and combination of form of bypass and preoperative disease stage.The results of our study suggest that previous and concomitant cardiovascular diseases and severity of the vascular disease itself should always be taken into account together with type of the graft and type of the operative procedure when planning femoropopliteal bypass in a patient with lower extremity vascular disease.

Keywords: *Femoropopliteal bypass, graft occlusion, risk factors.*

INTRODUCTION

Femoropopliteal bypass (FP) is relatively safe and effective technique of limb revascularization surgery for Chronic Critical Limb Ischaemia (CCLI) and disabling intermittent claudication (IC) in patients with peripheral arterial occlusive disease (PAOD). The initial outcome of this procedure is mostly good graft patency and limb preservation[1]. Although this surgical technique initially prolongs limb salvage, long-term graft patency is one of the major issues facing vascular surgeons[1] .

The most significant complication of FP bypass is graft occlusion, with frequency of 25 – 35% over a two-year postoperative period[1]. Graft occlusions are classified in three categories: early (<30 days), intermediate (<2 years) and late (>2 years)[2] . Limb salvage rate after the graft occlusion is poor, about 50% ± 5% over a two-year postoperative period [2].

According to the literature, FP bypass graft occlusion is associated with age[3], sex[4,5], race[3], preoperative hematocrit and hemoglobin values[3], clinical stage of disease [2,4,6,7], form of FP bypass[2,6], type of graft[4,8,9], number of crural recipient arteries patent at angiography[6,8,10,11], previous vascular intervention and progression of vascular disease[7,12-14], and type of anticoagulant (AC) and antiaggregant therapy (AA)[6,8,11]. On the other hand, there are conflicting published data about relevance of the following factors for the graft occlusion: associated chronic diseases in patients[3,15-17], drug therapy[3,13,15], perioperative blood transfusion, coagulation parameters (aPTT and INR)[18] , preoperative ankle systolic pressure index (ASPI)[19-23], alcohol and nicotine[3,7], Finnvastic score[24,25], lipid levels and statin therapy [15,26].

The aim of this study was to analyse insufficiently examined potential risk factors and risk factors with conflicting relevance according to prior studies, as well as their mutual interaction (possible synergistic effect of potential risk factors) and determination of their relative importance for FP bypass graft occlusions.

MATERIALS AND METHODS

Study design

The study design was of retrospective case-control type. It was conducted in patients with FP bypass graft occlusions which required surgical intervention, while the control group consisted of patients without such outcomes. Data were collected from the medical records of the patients. Risk factors for the occurrence of graft occlusion in patients with FP bypass were analyzed.

Study population

The study population consisted of the patients experiencing FP bypass surgery for advanced lower limb PAOD at Vascular Surgery Unit of the Clinical Centre Kragujevac, from January 2008. to December 2011. The cases were patients with FP bypass graft occlusions which required surgical intervention. The controls were patients without FP bypass graft occlusion after PAOD who were sex and age matched. The allocation ratio of the patients among groups was 1:2 in favor of controls. On the basis of the medical records of both groups it was observed to what extent they were exposed to the observed risk factors. This study did not include patients with incomplete medical records. The study was approved by the Ethics Committee of the Clinical Centre Kragujevac. The data were collected from the medical records of the patients.

The variables measured in the study

The main outcome of the study were FP bypass graft occlusions. The following potential risk factors for the graft occlusion were followed: age, sex, preoperative levels of hematocrit, hemoglobin, activated partial thromboplastin time (aPTT) and prothrombine time (PT/INR), clinical stage of disease according to Rutherford, number of angiographically patent crural recipient arteries, form of the FP bypass, type of the graft, previous vascular intervention, other patient's diseases (cardiovascular diseases, diabetes mellitus, chronic renal insufficiency, peptic ulcer disease, malignancy), drug therapy (angiotensin converting enzyme (ACE) inhibitors, alpha and beta blockers, calcium channel blockers, diuretics, metformin, insulin, anticoagulant and antiplatelet therapy), perioperative blood transfusion, smoking, alcohol consumption, and Finnvastic score.

Statistical analysis

In statistical data analysis continuous variables are presented as mean ± standard deviation (SD) in the text and tables, and categorical variables are presented as proportion of examined subjects with a particular outcome. Student's t-test was used for comparison of the mean values of continuous variables for small independent samples, and alternative non-parametric tests were used if the results did not follow normal distribution, based on the Kolmogorov-Smirnov test. Chi-squared (χ^2) test was used to compare differences in the frequencies of categorical variables, and Fisher's exact test was used if the frequency of categories was low. The influence of a number of variables on the examined dichotomous outcome (graft occlusions after FP bypass surgery), as well as the mutual interaction of potential predictor variables, were examined using binary logistic regression, and the results were presented as Adjusted Odds Ratio with corresponding 95% confidence intervals. All results where the probability of the null hypothesis being true was less than 5% ($p < 0.05$) were considered statistically significant. Statistical analysis was performed using a standard statistical software package (SPSS software V.18.0).

RESULTS

Basic characteristics of cases and controls and the differences between them are shown in Table I. There were no significant differences between cases and controls in terms of: sex, age, preoperative values of aPTT and INR, perioperative blood transfusion, ACE inhibitors, alpha and beta blockers, diuretics, calcium-channel blockers, metformin, insulin, diabetes, chronic renal insufficiency, peptic ulcer, malignancies, aorto-iliac occlusive disease, number of angiographically patent crural recipient arteries, the administration and type of antiaggregants and anticoagulants, alcohol consumption, duration of nicotine consumption, and Finnvastic score.

Statistically significant effects for the occurrence of FP bypass graft occlusion were found in the form of FP bypass (below-knee vs above-knee) ($OR_{adjusted}$ 115.34; 95% confidence interval 4.02,3306.55; p=0.006), graft type (artificial vs venous) ($OR_{adjusted}$ 81.62; 95% confidence interval 1.03,6486.32; p=0.049), associated cardiovascular diseases ($OR_{adjusted}$ 55.64; 95% confidence Interval 2.63,1176.94; p=0.010), previous vascular procedures ($OR_{adjusted}$ 51.61; 95% confidence interval 1.10,2425.14; p= 0.045), pre-operative disease stage according to Rutherford ($OR_{adjusted}$ 22.21; 95% confidence interval 2.62,188.39; p= 0.004), preoperative haemoglobin levels ($OR_{adjusted}$ 1.33; 95% confidence interval 1.01,1.74; p= 0.040) and preoperative hematocrit levels ($OR_{adjusted}$ 0.34; 95% confidence interval 0.13,0.89; p=0.028).

The results of logistic regression analysis (Coh & Snell R square 0.534, Nagelkerke R square 0.740, Hosmer-Lemeshow Chi-squared 4.713, df=8, p = 0.788) with adjustment for potential confounders are shown in Table II.

After examination of interaction between risk factors a significant synergistic effect was found for the combination of form of FP bypass and type of the graft ($OR_{adjusted}$ 140.95; 95%Confidence Interval 5.47,3632.14; p=0.003), type of the graft and previous vascular procedures ($OR_{adjusted}$ 75.96; 95%Confidence Interval 1.14, 5060.94; p=0.043), form of FP bypass and associated cardiovascular diseases ($OR_{adjusted}$ 68.03; 95% confidence interval 3.14,1472.69; p=0.007), type of graft and associated cardiovascular disease ($OR_{adjusted}$ 25.71; 95%Confidence Interval 1.69,391.30; p=0.019), type of graft and preoperative clinical stage of the disease according to Rutherford ($OR_{adjusted}$ 2.83; 95% Confidence Interval 1.28,6.24; p=0.010), form of FP bypass and preoperative clinical stage of the disease according to Rutherford ($OR_{adjusted}$ 2.58; 95 % confidence interval 1.47,4.51; p=0.001) (Table III).

Table I. Baseline characteristics of cases and controls

Variable		Cases (n=35)	Controls (n=70)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
Sex(M/F)		27/8 (77%/23%)	53/17 (76%/24%)	$\chi^2 = 0.26$ p = 0.871	1.08 (0.41, 2.83)
Age (years, mean ± SD)		65.00 ± 7.92	63.79 ± 10.02	T = 0.626 p = 0.533	1.01 (0.97, 1.06)
Clinical stage of disease according to Rutherford	I	0 0%	7 10%	$\chi^2 = 25.848$ p < 0.001*	2.82 (1.77, 4.48)
	II	0 0%	16 22%		
	III	6 17%	21 30%		
	IV	11 31%	13 19%		
	V	15 43%	13 19%		
	VI	3 9%	0 0%		
Duration of smoking (years, mean ± SD)		28.03 ± 16.08	26.51 ± 17.03	U=1213,000 p = 0.934	1.01 (0.98, 1.03)
Alcohol consumption		8/27 (23%/77%)	18/52 (26%/74%)	$\chi^2 = 0.102$ p = 0.749	0.86 (0.33, 2.22)
Form of femoropopliteal bypass(below-knee vs above-knee)		25/10	22/48	$\chi^2 = 15.099$	5.45 (2.24, 13.28)

Variable	Cases (n=35)	Controls (n=70)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
	(71%,29%)	(31%, 69%)	p < 0.001*	
Anticoagulant(AC) and Antiaggregant (AA) therapy	AC+AA 23 66% AC 11 31% AA 0 0% without AA/AC 3 3%	AC+AA 34 49% AC 29 41% AA 4 6% without AA I AC 3 4%	χ^2 =4.001 p = 0.261	0.58 (0.31, 1.11)
Type of heparin	SH 28 80% LMWH 5 14% S+LMWH 2 6%	S 54 77% LMWH 10 14% S+LMWH 6 9%	χ^2 = 0.274 p = 0.872	0.85 (0.42, 1.71)
Preoperative levels of haemoglobin	128.91 ± 20.85	133.67 ± 19.98	T = -1.134 p = 0.260	0.99 (0.97, 1.01)
Preoperative levels of aPTT	28.12 ± 5.11	27.92 ± 6.27	U = 1157.500 p = 0.646	1.01 (0.94, 1.08)
Preoperative levels of INR	1.14 ± 0.30	1.08 ± 0.38	U = 1067.000 p = 0.283	1.57 (0.51, 4.89)
Preoperative levels of hematocrit	38.57 ± 6.36	40.18 ± 5.67	T = -1.323 p = 0.189	0.95 (0.891, 1.023)
Aortoiliac stenosis	6/29 (17%/83%)	18/52 (26%/74%)	χ^2 = 0.972 p = 0.324	0.60 (0.21, 1.67)
Previous vascular intervention	9/26 (26%/74%)	13/57 (19%/81%)	χ^2 = 0.719 p = 0.397	1.52 (0.58, 4.00)
Angiotensin converting enzyme (ACE) inhibitors	24/11 (69%/31%)	50/19 (71%/29%)	χ^2 = 0.677 p = 0.713	0.80 (0.33, 1.94)
Beta blockers	16/19 (46%/54%)	28/42 (40%/60%)	χ^2 = 0.313 p = 0.576	1.26 (0.56, 2.86)
Diuretics	11/24 (31%/69%)	24/46 (34%/66%)	χ^2 = 0.086 p = 0.770	0.88 (0.37, 2.09)
Calcium channel blockers	6/29 (17%/83%)	17/53 (24%/76%)	χ^2 = 0.696 p = 0.404	0.64 (0.23, 1.82)
Metformin	2/33 (6%/94%)	14/56 (20%/80%)	χ^2 = 3.687 p = 0.055	0.24 (0.05, 1.13)
Insulin	5/30 (14%/86%)	9/61 (13%/87%)	χ^2 = 0.041 p = 0.839	1.13 (0.35, 3.67)
Type of graft (artificially vs venous)	32/3 (91%/9%)	61/9 (87%/13%)	χ^2 = 0.423 p = 0.515	1.57 (0.40, 6.22)
Finnvastic score	5/30 (14%/86%)	5/65 (7%/93%)	χ^2 = 1.382 p = 0.295	2.17 (0.58, 8.05)
Cardiovascular diseases	20/15 57%/43%	26/44 37%/63%	χ^2 = 3.791 p = 0.052	2.26 (0.98, 5.16)
Chronic renal insufficiency	5/30 14%/86%	4/66 6%/94%	χ^2 = 2.188 p = 0.156	2.75 (0.69, 10.97)
Peptic ulcer disease	5/30 14%/86%	5/65 7%/93%	χ^2 = 1.382 p = 0.295	2.17 (0.58, 8.05)
Number of angiographically passable crural recipient arteries 1/2/3	16/9/8 48,5%/27,3%/24,2%	15/28/21 32,8%/43,8%/23,4%	χ^2 = 6.359 p = 0.042*	1.75 (1.01, 3.06)

Variable	Cases (n=35)	Controls (n=70)	Test value and significance of null hypothesis	Crude odds ratios with confidence intervals (1.96*SE)
Diabetes	11/24 31%/69%	23/47 33%/67%	$\chi^2 = 0.022$ p = 0.883	0.94(0.39, 2.24)
Malignancy	0/35 0%/100%	5/65 7%/93%	$\chi^2 = 2.625$ p = 0.167	0.00
Perioperative blood transfusion	6/29 17%/83%	9/61 13%/87%	$\chi^2 = 0.350$ p = 0.554	1.40 (0.46, 4.31)
Alpha blockers	2/33 6%/94%	2/68 3%/97%	$\chi^2 = 0.520$ p = 0.471	2.06 (0.28, 15.28)

* Significant difference
SD= Standard Deviation
AC =Anticoagulants
AA = Antiaggregant
SH = Standard heparin
LMWH = Low molecular weight heparin
aPTT = Activated Partial Thromboplastin Time;
INR = international Normalized Ratio;
ACE = Angiotensin Converting Enzyme;

Table II. Crude and adjusted odds ratios of the risk factors for femoropopliteal bypass graft occlusions*

Risk factors	Crude OR (95% CI)	Adjusted OR (95% CI)
Form of femoropopliteal bypass(below-knee vs above-knee)	5.45(2.24, 13.28)	115.34(4.02, 3306.55)
Type of graft (artificially vs venous)	1.57(0.40, 6.22)	81.62(1.03, 6486.32)
Cardiovascular diseases	2.26(0.98, 5.16)	55.64(2.63, 1176.94)
Previous vascular intervention	1.52(0.58, 4.00)	51.61(1.10, 2425.14)
Clinical stage of disease according to Rutherford	2.82(1.77, 4.48)	22.21(2.62, 188.39)
Preoperative levels of haemoglobin	0.99(0.97, 1.01)	1.33(1.01, 1.74)
Preoperative levels of hematocrit	0.95(0.89, 1.02)	0.34 (0.13, 0.98)

* For the sake of clarity only significant associations are shown in the table (95% CI of adjusted OR does not include value of 1)
OR = Odds Ratio;
CI = onfidence Intervals

Table III. Synergistic effects of risk factors for the femoropopliteal bypass graft occlusions

Risk factors	Crude OR (95% CI)	Adjusted OR (95% CI)
Form of femoropopliteal bypass (below-knee vs above-knee)	5.45(2.24, 13.28)	115.34(4.02, 3306.55)
Type of graft (artificially vs venous)	1.57(0.40, 6.22)	81.62(1.03, 6486.32)
Cardiovascular diseases	2.26(0.98, 5.16)	55.64(2.63, 1176.94)
Previous vascular intervention	1.52(0.58, 4.00)	51.61(1.10, 2425.14)
Clinical stage of disease according to Rutherford	2.82(1.77, 4.48)	22.21(2.62,188.39)
Preoperative levels of haemoglobin	0.99(0.97, 1.01)	1.33(1.01, 1.74)
Preoperative levels of hematocrit	0.95 (0.89, 1.02)	0.34 (0.13, 0.98)

Risk factors	Crude OR (95% CI)	Adjusted OR (95% CI)
Form of femoropopliteal bypass(below-knee vs above-knee) by Type of graft (artificially vs venous)	5.54(2.30,13.35)	140.95(5.47, 3632.14)* p=0.003
Type of graft (artificially vs venous) by Previous vascular intervention	1.50(0.52,4.35)	75.96(1.14, 5060.94)* p=0.043
Cardiovascular diseases by Form of femoropopliteal bypass(below-knee vs above-knee)	4.50(1.75,11.60)	68.03(3.14, 1472.69)* p=0.007
Type of graft (artificially vs venous) by Cardiovascular diseases	2.03(0.89,4.64)	25.71(1.69, 391.30)* p=0.019
Previous vascular intervention by Form of femoropopliteal bypass(below-knee vs above-knee)	2.70(0.76,9.53)	4.22(0.20, 90.46) p=0.357
Type of graft (artificially vs venous) by Clinical stage of disease according to Rutherford	1.86(1.39,2.61)	2.83 (1.28, 6.24)* p=0.010
Clinical stage of disease according to Rutherford by Form of femoropopliteal bypass(below-knee vs above-knee)	1.65(1.32,2.05)	2.58(1.47, 4.51)* p=0.001
Preoperative levels of haemoglobin by Form of femoropopliteal bypass(below-knee vs above-knee)	1.01(1.01,1.02)	1.02(1.01,1.04) p=0.008
Preoperative levels of hematocrit by Form of femoropopliteal bypass(below-knee vs above-knee)	1.04(1.02,1.06)	1.06(1.02,1.12) p=0.009
Preoperative levels of haemoglobin by Preoperative levels of hematocrit	1.00(1.00-1.00)	1.00(0.99,1.00) p=0.381

* Clinically significant interaction
OR = Odds Ratio
CI = Confidence Interval

DISCUSSION

Each year about 220 new cases of CCLI per million are reported worldwide[16]. Revascularization procedures are necessary in approximately 50% of patients and FP bypass is usually performed[16] . A major complication after FP bypass surgery is graft occlusion with a frequency of 25 – 35% over a two-year postoperative period[1].

Since previous studies demonstrated the impact of gender[4,5,7] and age[3] on the occurrence of graft occlusions, we matched our patients by sex and age in order to discover influences of other variables. According to our research main risk factors for graft occlusion are the form of FP bypass, type of graft, associated cardiovascular disease, previous vascular procedures and preoperative clinical stage of the disease according to Rutherford.

Below-knee FP bypass was a significant risk factor for graft occlusion in our study, and these results are consistent with other studies[3,7]. In addition, below-knee FP bypass is a significant factor affecting the limb salvage duration[2].

Type of graft, i.e. synthetic graft, is an important risk factor for graft occlusion according to the results of our study, which corresponds with the literature explaining a clear benefit for autologous vein when compared to synthetic materials[4,7-9,13,27]. Also, limb ischaemia resulting from graft occlusion seemed to cause more severe effects in artificial graft compared with venous graft[8,27]. Autogenous saphenous vein is considered to be the best material for FP bypass. A synthetic graft is considered only as an acceptable alternative. There is a significantly increased rate of occlusion in artificial graft compared to venous graft (48% versus 18% ; p =0.005)[8] .

Interaction between the type of graft and form of bypass showed highly significant association with graft occlusion and these results also correspond to previous research[3]. According to these results synthetic peripheral FP bypass seemed to have the highest rate of graft occlusion, as shown in other studies, where early graft occlusion was around 8.2% in artificial graft and 4.7% in vein graft[3] .

However, according to previous studies impact of patients' chronic diseases on graft occlusion is a conflicting issue[15,28]. We found a significant impact of associated cardiovascular diseases on graft occlusion. It is logical, since coronary artery disease and cerebrovascular disease often appear along with PAOD as a manifestation of generalized atherosclerosis. Approximately 40-60% of patients with PAOD also have associated cardiovascular disease[16]. In patients with cardiovascular disease peripheral perfusion is reduced due to heart failure and left ventricular relaxation. Consequently, in patients with PAOD and related cardiovascular diseases graft occlusions occur more frequently. On the contrary, in some studies patients with diabetes have a lower

incidence of graft occlusion, and better results after FP bypass procedures if compared with patients without diabetes[3]. There is also an inverse association between diabetes and graft occlusion after FP bypass, but without statistical significance[3]. The rate of early occlusion in non-diabetic patients was 5.5%, in insulin-dependent diabetic patients 4.4%, and in patients with insulin-independent diabetes 4.0%[3]. The occurrence of early occlusion was 28% less likely in patients who take insulin than in those who take oral antidiabetics[3]. However, in our study we did not find significant effect of diabetes and type of antidiabetic drug on graft occlusion. This is not surprising, since in patients with diabetic PAOD is more aggressive, with early major blood vessels involvement and more rapid disease progression.

There is also controversy over significance of the impact of chronic renal insufficiency (HRI) in patients with PAOD on graft occlusion after FP bypass. Some authors state that HRI has no effect on graft occlusion, which corresponds to our results[17]. There are also dissenting opinions[13]. Since the mortality rate of these patients is already high, it is important to identify the subset of patients who demonstrate benefit and improve the quality of life after revascularization.

Our results also showed statistical significance of previous vascular and endovascular intervention in relation with graft occlusion. They indicate generalized atherosclerosis and more advanced stages of the disease. According to some studies previous aortobifemoral bypass, contralateral amputation, ipsilateral FP bypass, grafting revision, and endovascular procedures are significant risk factors for graft occlusion[13,14] .

Advanced clinical stage of disease according to Rutherford as a manifestation of advanced limb atherosclerosis and advanced vascular ischaemia, according to our results, significantly affect the occurrence of graft occlusion, which corresponds with the results of other studies[2,6,7]. In patients who underwent FP bypass for claudication, pain at rest or tissue loss, we found significantly lower limb preservation rates[2] .

According to the literature average hematocrit level in the examined patients is 38%. In patients with higher hematocrit level the risk of graft occlusion is lower if compared to those with lower hematocrit levels[3]. The decrease by 1% below 38% hematocrit level increases the rate of occlusion for 2%[3]. Our results indicate that hematocrit levels are inversely proportional to occurrence of occlusion, which corresponds to the results of other authors[3]. However, although statistically significant, hematocrit level may not be clinically relevant.

According to most authors number of patent crural recipient arteries significantly affects the occlusion[1,10]. We did not find a number of patent crural recipient arteries to be statistically significant factor, which was also found by some authors[22] .

Nicotine consumption is an influential factor. A number of authors emphasize that smoking is significant factor in the development of graft occlusion[7] , while others dispute it as a statistically significant effect[3]. We did not find that duration of nicotine consumption influences graft occlusion.

The results obtained in our study did not show significant impact of AA and AC therapy on the occurrence of graft occlusion, which corresponds to previous results[8]. The prevention of postoperative graft occlusion by AC and AA, or by combination therapy (AA and AC) is a routine measure. In prevention of polytetrafluoroethylene (PTFE) graft occlusion there was a slight but not statistically significant decrease in the frequency of occlusion using AC and AA therapy if compared to AA (39.3% versus 47.2%; $p = 0.62$)[8]. Two-fold increase in risk of severe ischemia without the use of AC vitamin K antagonist (VKA) indicated that, even not statistically significant, AC VKA and AA therapy was useful in PTFE graft[8]. Given the increased risk of bleeding and costly treatment, PTFE graft may be considered inappropriate for FP bypass, if great saphenous vein is available[8] .

Some authors state that AC VKA may be useful for FP bypass with low graft flow (midgraft velocity $\leq$ 45 cm/s). Given the low incidence of high-flow bypass occlusion, the use of AC therapy was not significant for either popliteal or femoral bypass[6]. Although not statistically significant, AC therapy is justified considering the two-fold lower risk of severe limb ischaemia[8]. Aspirin has a slight effect on the patency of the graft, but such effect was significantly lower in venous graft when compared to artificial graft[11] .

Curi and associates show reduced long-term graft patency in patients with hypercoagulable states[18]. They are usually at young age. We have not found a significant effect of preoperative coagulation factors on graft occlusion.

The main drawback of this study were its limitation to one centre and its retrospective design. Although we used some of the useful strategies for minimizing potential subjectivity in sampling and measuring the outcomes, such as matching of cases with controls, a lot of patients were not included due to incomplete patient files, and true randomization of matched controls was not possible. These circumstances hampered our ability to analyze the impact of some potentially important factors on the occurrence of graft occlusion after FP bypass, such as body mass index , blood lipid parameters and hypolipemic therapy, primarily statins, and color duplex scan verified postoperative graft blood flow velocity.

CONCLUSIONS

The results of our study suggest that previous and concomitant cardiovascular diseases and severity of the vascular disease itself should always be taken into account together with type of the graft and type of the operative procedure when planning femoropopliteal bypass in a patient with lower extremity vascular disease. Adequate indications for surgery, preoperative assessment and preparation of patients

with chronic conditions, selecting the form of bypass and type of graft and dealing with preventable risk factors significantly should reduce the rate of graft occlusions, limb amputations and disabled patients. This study may also suggest some hypotheses for future research, in which well known risk factors of graft occlusion after femoropopliteal bypass may be combined with the certain factors identified in this survey as significant predictors of the graft occlusion.

ACKNOWLEDGEMENTS

This study was partially financed by the grant No 175007 given by the Serbian Ministry of Education and by the grant No 01-404 given by the Ministry of Science, Montenegro.

REFERENCES

1. Pulli R, Dorigo W, Castelli P, Dorrucci V, Ferilli F, De Blasis G et al. Midterm results from a multicenter registry on the treatment of infrainguinal critical limb ischemia using a heparin-bonded ePTFE graft. J Vasc Surg 2010;51(5) :1167-77.
2. Baldwin ZK, Pearce BJ, Curi MA, Desai TR, McKinsey JF, Bassiouny HS *et al.* Limb salvage after infrainguinal bypass graft failure. J Vasc Surg 2004; 39: 951–7.
3. Singh N, Sidawy AN, DeZee KJ, Neville RF, Akbari C, Xenderson W. Factors associated with early failure of infrainguinal lower extremity arterial bypass. J Vasc Surg 2008;47:556–61.
4. Tangelder MJ, Algra A, Lawson JA, Eikelboo m BC. Risk factors for occlusion of infrainguinal bypass grafts. Eur J Vasc Endovasc Surg 2000; 20(2):118-24.
5. Alpagut U, Ugurlucan M, Banach M, Mikhailidis DP, Dayioglu E.Does gender influence the patency of infrainguinal bypass grafts?Angiology 2008; 59(3):278-82.
6. Brumberg RS, Back MR, Armstrong PA, Cuthbertson D, Shames ML, Johnson BL et al. The relative importance of graft surveillance and warfarin therapy in infrainguinal prosthetic bypass failure. J Vasc Surg 2007;46(6):1160-6.
7. Lancaster RT, Conrad MF, Patel VI, Cambria RP, LaMuraglia GM. Predictors of early graft failure after infrainguinal bypass surgery: a risk-adjusted analysis from the NSQIP. Eur J Vasc Endovasc Surg 2012;43(5): 549-55.
8. Jackson MR, Johnson WC, Williford WO, Valentine RJ, Clagett GP. The effect of anticoagulation therapy and graft selection on the ischemic consequences of femoropopliteal bypass graft occlusion: results from a multicenter randomized clinical trial. J VascSurg 2002; 35(2):292-8.
9. Schanzer A, Hevelone N, Owens CD, Belkin M, Bandyk DF, Clowes AW et al.Technical factors affecting autogenous vein graft failure: observations from a large multicenter trial. J Vasc Surg 2007;46(6):1180-90.
10. Robinson KD, Sato DT, Gregory RT, Gayle RG, DeMasi RJ, Parent FN *et al.* Long-term outcome after early infrainguinal graft failure. J Vasc Surg 1997;26:425-438.
11. Brown J, Lethaby A, Maxwell H, Wawrzyniak AJ, Prins MH. Antiplatelet agents for preventing thrombosis after peripheral arterial bypass surgery.Cochrane Database Syst Rev 2008;8(4):CD000535
12. Bosiers M, Deloose K, Verbist J, Schroë H, Lauwers G, Lansink W et al. Heparin-bonded expanded polytetrafluoroethylene vascular graft for femoropopliteal and femorocrural bypass grafting: 1-year results. J Vasc Surg 2006;43(2):313-8.
13. Nolan BW, De Martino RR, Schanzer A, Goodney PP, Walsh DW, Cronenwett JL. Prior failed ipsilateral percutaneous endovascular intervention in patients with critical limb ischemia predicts poor outcome after lower extremity bypass. J Vasc Surg 2011;54(3):730-5.
14. Baril DT, Goodney PP, Robinson WP, Nolan BW, Stone DH, Li Y et al. Prior contralateral amputation predicts worse outcomes for lower extremity bypasses performed in the intact limb. J Vasc Surg 2012;56(2):353-60.
15. Henke PK, Blackburn S, Proctor MC, Stevens J, Mukherjee D, Rajagopalin S et al. Patients undergoing infrainguinal bypass to treat atherosclerotic vascular disease are underprescribed cardioprotective medications: effect on graft potency, limb salvage, and mortality. J Vasc Surg 2004;39(2):357-65.
16. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG et al. Inter-society consensus for the management of peripheral arterial disease (TASC II). Eur J Vasc Endovasc Surg 2007;33(Suppl 1):1–75.
17. Biancari F, Arvela E, Korhonen M, Söderström M, Halmesmäki K, Albäck A et al.End-stage renal disease and critical limb ischemia:a deadly combination? Scand J Surg 2012; 101(2): 138–43.
18. Curi MA, Skelly CL, Baldwin ZK, Woo DH, Baron JM, Desai TR et al.Long-term outcome of infrainguinal bypass grafting in patients with serologically proven hypercoagulability. J Vasc Surg 2003;37(2): 301–6.
19. Rutherford RB, Jones DN, Bergentz SE,Berqqvist D, Comerota AJ, Dardik H et al. Factors affecting the patency of infrainguinal bypass. J Vasc Surg 1988;8:236-46.
20. Kalra M, Gloviczki P, Bower TC, Panneton JM, HarmsenWS, Jenkins GD et al. Limb salvage after successful pedal bypass grafting is associated with improved long-term survival. J Vasc Surg 2001; 33(1):6-16.
21. Dean RH, Yao JS, Stanton PE, Bergan JJ. Prognostic indicators in femoropopliteal reconstructions. Arch Surg 1975;110(11):1287-93.
22. Dolgin C, Collins R, Martin E, Voorhees AB, Nowygrod R.The prognostic value of the noninvasive vascular laboratory in autologous vein bypasses of the lower extremities. J Cardiovasc Surg (Torino) 1983;24(3):231-4.
23. Corson JD, Johnson WC, LoGerfo FW, Bush HL Jr, Menzoian JO, Kumaki DJ et al. Doppler ankle systolic blood pressure. Prognostic value in vein bypass grafts of the lower extremity. Arch Surg 1978;113(8):932-5.
24. Arvela E, Söderström M, Korhonen M, Halmesmäki K, Albäck A, Lepäntalo M et al. Finnvasc score and modified Prevent III score predict long-term outcome after infrainguinal surgical and endovascular revascularization

for critical limb ischemia. J Vasc Surg 2010;52(5):1218-25.
25. Kechagias A, Ylönen K, Kechagias G, Juvonen T, Biancari F. Limits of infrainguinal bypass surgery for critical leg ischemia in high-risk patients (Finnvasc score 3-4). Ann Vasc Surg 2012;26(2):213-8.
26. Abbruzzese TA, Xavens J, Belkin M, Donaldson MC, Whittemore AD, Liao JK et al. Statin therapy is associated with improved patency of autogenous infrainguinal bypass graft. J Vasc Surg 2004;39(6):1178-85.
27. Twine CP, McLain AD. Graft type for femoro-popliteal bypass surgery. Cochrane Database Syst Rev 2010;(5):CD001487.
28. Seeger JM, Pretus HA, Carlton LC, Flynn TC, Ozaki CK, Huber TS. Potential predictors of outcome in patients with tissue loss who undergo infrainguinal vein bypass grafting. J Vasc Surg 1999;30:427–35.
29. Berceli SA, Chan AK, Pomposelli FB Jr,Gibbons GW, Campbell DR, Akbari CM et al. Efficacy of the dorsal pedal artery bypass in limb salvage for ischemic heel ulcers. J Vasc Surg 1999; 30:499-508.

MODULATORY ROLE OF GALECTIN-1 IN ULCERATIVE COLITIS WITH COMORBID METABOLIC SYNDROME

Kemal Corovic[1], Bojan Stojanovic[2], Andjela Petrovic[3], Isidora Stanisavljevic[3], Veljko Maric[4], Natasa Zdravkovic[5] and Marina Jovanovic[5]

[1] *Health Center Tutin, Serbia*
[2] *University of Kragujevac, Faculty of Medical Sciences, Department of Surgery, Kragujevac, Serbia*
[3] *University of Kragujevac, Serbia, Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research*
[4] *University of East Sarajevo, BiH, Medical faculty, Department of Surgery*
[5] *University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Internal medicine*

Received: 25.05.2023.
Accepted: 17.07.2023.

Corresponding author:

Bojan Stojanovic

University of Kragujevac, Faculty of Medical Sciences, Department of Surgery, Kragujevac, Serbia

E-mail: bojan.stojanovic01@gmail.com

ABSTRACT

Metabolic syndrome (MetS) and ulcerative colitis (UC) are widespread health conditions characterized by chronic, low-grade inflammation. Galectin-1 (Gal-1), an immunomodulatory peptide mainly secreted from adipose tissue, could potentially play a crucial role in mitigating these conditions. This cross-sectional study explores the involvement of Gal-1 in MetS and UC within a cohort of 75 patients, newly diagnosed with UC. The MetS subgroup displayed increased fecal Gal-1 levels compared to those without MetS. Furthermore, Gal-1 showed predominance over pro-inflammatory cytokines, including TNF-α, IL-6, and IL-17, in these subjects. These findings emphasize the potential involvement of Gal-1 in the pathophysiology of UC and MetS, presenting it as a promising diagnostic biomarker and therapeutic target for these conditions.

Keywords: *Galectin-1, Ulcerative Colitis, Metabolic Syndrome, Inflammation.*

UDK:

Eabr 2026; 27(2):135-141
DOI:10.2478/eabr-2023-0006

INTRODUCTION

Ulcerative Colitis and Metabolic Syndrome are two disparate medical conditions, both representing significant burdens on global health (1). UC, an inflammatory bowel disease, primarily affects the colon, leading to chronic inflammation and ulcers (2). MetS, on the other hand, is a cluster of conditions - increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol levels - that occur together, increasing the risk of heart disease, stroke, and type 2 diabetes (3).

Galectin-1 (Gal-1), a cell-surface binding lectin protein, is involved in numerous biological processes, including inflammation regulation, immune response modulation, cell-cell adhesion, and cell growth (4). This protein is notably abundant in inflamed tissues, and its secretion intensifies in activated macrophages within these areas (5). In patients with UC, characterized by mild colonic tissue inflammation, serum Gal-1 levels have been observed to significantly rise compared to those with severe inflammation (6). This discrepancy suggests a protective role for Gal-1 in preserving the intestinal epithelium.

Paralleling these observations in UC, MetS – a condition marked by chronic low-grade inflammation, insulin resistance, and obesity – also reveals intriguing connections with Gal-1 (7). This protein is implicated in managing inflammation within adipose tissue and moderating insulin resistance, both crucial elements in MetS pathophysiology (8). Increased systemic Gal-1 levels found in metabolic diseases underline its potential role in the genesis of these disturbances (9). Taken together, these findings highlight the potential pivotal role of Gal-1 in the pathophysiological mechanisms that underlie both UC and MetS. This suggests that Gal-1 could be a promising novel biomarker for these conditions.

The intersection between UC and MetS warrants further exploration. Patients diagnosed with UC are at a heightened risk of developing MetS, and reciprocally, those with MetS have a greater predisposition towards UC (10). Chronic inflammation, a shared trait in both conditions, might play a key role in the interrelation between UC and MetS. Given its function in managing inflammation and metabolic processes, Gal-1 could serve as a critical connector between these two pathologies.

Therefore, this study aims to delve into the relationship between systemic and local Gal-1 levels in individuals with UC and MetS. By doing so, we aim to contribute to a more profound understanding of the role of Gal-1 in inflammation and immune modulation in these conditions. Our findings may provide novel insights into potential therapeutic approaches for UC and MetS by targeting the inflammatory pathway via modulation of Gal-1.

MATERIALS AND METHODS

Characteristics of the Study Population

A total of 75 patients (42 males and 33 females), aged between 21 and 80 years, with newly diagnosed and histologically confirmed ulcerative colitis, were enrolled in an observational cross-sectional study. Recruited UC patients were divided in two groups, utilizing the Adult Treatment Panel III (ATP III) criteria for the diagnosis of MetS (11): Group without MetS (no MetS) and group with MetS. The criteria for diagnosing metabolic syndrome (MetS) according to the ATP III guidelines include the presence of three or more of the following components: 1)Central Obesity: Defined by waist circumference measurements. A waist circumference exceeding 40 inches (102 cm) in men and 35 inches (88 cm) in women is indicative of central obesity; 2) Hypertension: Characterized by blood pressure readings of 130/85 mmHg or higher, or active treatment for previously diagnosed hypertension; 3) Dysglycemia: Noted by a fasting plasma glucose level exceeding 5.5 mmol/L (99 mg/dL) and/or a 2-hour post-load plasma glucose level above 7.8 mmol/L (140 mg/dL), or active treatment for previously diagnosed dysglycemia; 4) Elevated Triglycerides: Triglyceride levels at or above 1.7 mmol/L (150 mg/dL) are considered high, 5) Low High-Density Lipoprotein (HDL) Cholesterol: Levels below 1.03 mmol/L (40 mg/dL) in men and 1.29 mmol/L (50 mg/dL) in women are deemed low, indicating potential metabolic imbalance.

All patients within the UC + MetS group meet all of the aforementioned criteria. Each patient received an exhaustive medical evaluation comprising a thorough medical history review, physical examination, routine laboratory tests, and diagnostic imaging procedures, including chest X-ray, abdominal ultrasound, abdominal computed tomography scan, and endoscopy. The research took place at two premier institutions - the Center for Gastroenterology, Clinical Center of Kragujevac, and the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia. Ethical approval was secured from the respective Ethic Committee of Clinical Center of Kragujevac. The study rigorously upheld the Principles of Good Clinical Practice and the Declaration of Helsinki. Informed consent was obtained from all patients before conducting blood and tissue analyses. Throughout the study, patients received continuous medical supervision at the Clinical Center Kragujevac, ensuring optimal care and safety.

The exclusion criteria for this study were as follows: Individuals under the age of 21 or over the age of 80, pregnant or breastfeeding women, patients with a history of other inflammatory bowel diseases such as Crohn's disease, individuals previously diagnosed with metabolic conditions other than Metabolic Syndrome, such as Type 1 diabetes or Cushing's syndrome. Additionally, those with a history of cancer, severe cardiovascular diseases, liver or renal insufficiency, or any autoimmune diseases were excluded. Patients who had undergone bowel resection surgery or had a stoma were also

excluded. Finally, individuals who had used immunosuppressants, systemic corticosteroids, or antibiotics within the past 3 months, or were participating in other clinical trials, were also excluded from the study.

The diagnosis and severity assessment of UC in each case were established based on histological and clinical scores. To ensure consistent evaluation of the mucosal condition, all endoscopic procedures were conducted by a skilled endoscopist. The severity of endoscopic lesions in UC was determined using the Mayo endoscopic sub-score (12). This scoring system assesses the extent and severity of inflammation observed during endoscopy. It includes various criteria such as vascular pattern, erythema, friability, and ulceration. The scoring ranges from 0 to 3, with 0 indicating no inflammation and 3 representing severe inflammation.

The clinical score for assessing the severity of UC was determined using two indices: the Truelove and Witts clinical activity index and the Mayo clinical index (13). The Truelove and Witts clinical activity index evaluates various clinical parameters such as the number of stools per day, presence of blood in the stool, temperature, pulse rate, and general well-being. Each parameter is assigned a score, and the cumulative score provides an overall assessment of disease activity. On the other hand, the Mayo clinical index incorporates additional factors such as physician's global assessment, patient-reported outcomes, and laboratory markers like erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels. This index aims to provide a comprehensive evaluation of the patient's clinical status, considering both objective measures and subjective assessments.

The histological score in this study was determined using the Geboes grading system (14). Histological sections were analyzed in a blinded manner by two independent pathologists. This approach ensured unbiased evaluation of the histological features without knowledge of other clinical or endoscopic findings. The Geboes grading system provides a standardized framework for assessing histopathological changes in ulcerative colitis, including parameters such as inflammation, epithelial damage, crypt architecture, and presence of ulceration.

Quantification of Fecal and Serum Cytokine Levels

The procedure for preparing fecal samples followed the method outlined earlier (15). To summarize, a 1g sample of feces was diluted, stirred, and homogenized in 5mL of a protease inhibitor cocktail (P8340, Sigma Aldrich, St. Louis, MO, USA). Blood samples were collected from both patients and control subjects at 8 am, with serum being separated, gathered, and preserved at -80 °C until required for testing. The serum and fecal supernatants from UC patients were assessed for levels of tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), interleukin-10 (IL-10), interleukin-17 (IL-17), and Gal-1. The quantification was carried out using ELISA tests commercially available, following the guidelines provided by the product manufacturer.

Statistical analysis

All statistical analyses in this study were performed using SPSS software (version 22.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize participant demographics and clinical characteristics. Continuous variables were presented as mean ± standard error of mean (SE) based on their distribution, assessed by the Shapiro-Wilk test. Categorical variables were reported as frequencies and percentages. The Student's t-test (for normally distributed data) or Mann-Whitney U test (for non-normally distributed data) were used to compare continuous variables between two groups. Categorical variables were compared using the Chi-square test or Fisher's exact test, as appropriate. A p-value <0.05 was considered statistically significant in all analyses.

RESULTS

Laboratory Parameter Differences between UC Patients with and without Metabolic Syndrome

A total of 75 patients were enrolled in the study, of which 61 had Ulcerative Colitis (UC) with Metabolic Syndrome (MetS) and 14 had UC without MetS. The study revealed significant differences in certain laboratory parameters between these two patient groups. Patients with UC and MetS demonstrated significantly elevated levels of creatinine (82.21 ±2.89 μmol/L vs 66.75 ±3.54 μmol/L, p=0.014) and urea (6.17 ± 0.42 mmol/L vs 3.65 ± 0.54 mmol/L, p<0.05) compared to UC patients without MetS. Furthermore, the liver enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) also showed significantly higher values in UC patients with MetS (AST: 33.51± 2.57 U/L vs 22.74 ±2.27 U/L, p=0.03; ALT: 37.86±6.21 U/L vs 22.69 ± 3.39 U/L, p= 0.031). Additionally, serum triglycerides (1.72 ± 0.21 mmol/L vs 0.79 ± 0.09 mmol/L, p= 0.003) and cholesterol levels (5.22 ± 0.17 mmol/L vs 3.63 ± 0.25 mmol/L, p= 0.001) were significantly higher in patients with both UC and MetS as compared to those with UC alone. In contrast, the leukocyte count was found to be significantly lower in UC and MetS patients (8.09 ± 0.25 $x10^9$/L vs 10.57 ± 1.34 $x10^9$/L). However, there were no significant differences between the two groups regarding the number of thrombocytes, hemoglobin levels, HDL, Low-Density Lipoprotein (LDL), Gamma-Glutamyl Transferase (gamma GT), albumin, and globulin levels (data not shown).

Severity of UC Manifestations in Patients with Metabolic Syndrome

Patients diagnosed with both Metabolic Syndrome (MetS) and Ulcerative Colitis (UC) displayed significantly milder manifestations, despite elevated metabolic markers. This was evidenced by a notably lower Mayo endoscopic subscore (3±0 vs 2±0.75; p=0.038) and Mayo clinical score (3±0 vs 2±1; p=0.005). Endoscopic examination typically showed minor mucosal abnormalities, in contrast to more severe findings in UC patients without MetS. Pathohistological scores, such as chronic inflammatory infiltration (2.20±0.50

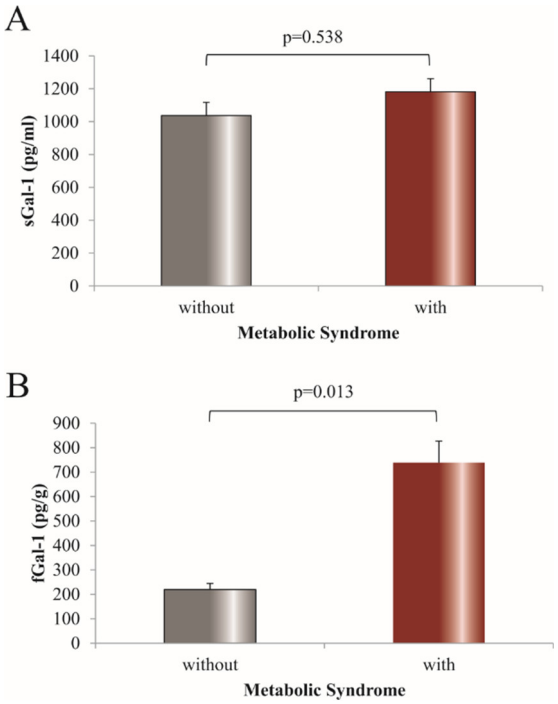
vs 1.75 ± 0.20; p = 0.044) and eosinophilic infiltration (2.10 ± 0.50 vs 1.50± 0.25; p = 0.031), further supported these observations, suggesting that MetS might attenuate UC severity.

Elevated Galectin-1 Levels and Its Ratio with Pro-Inflammatory Markers in Sera and Fecal Samples of UC Patients with Metabolic Syndrome

We evaluated the concentration of Gal-1 in both the sera and fecal liquid fraction across all UC patients. From previous research, it was demonstrated that patients with MetS displayed a significantly lower serum level of the pro-inflammatory cytokine IL-17 (16). Yet, in the same study, there were no significant differences in systemic concentrations of pro-inflammatory cytokines such as TNF-α and IL-6 between patients with or without MetS. Furthermore, fecal concentrations of these pro-inflammatory cytokines did not significantly differ between the two groups (16).

As illustrated in Figure 1A, no notable difference was observed in systemic concentration of Gal-1 between patients with and without MetS (181.4±110.7 pg/ml vs 1037.0±80.0 pg/ml; p= 0.538). Conversely, a significantly higher level of fecal Gal-1 was noted in MetS patients (738.78±88.02 vs 219.07±25.39; p= 0.013) (Figure 1B).

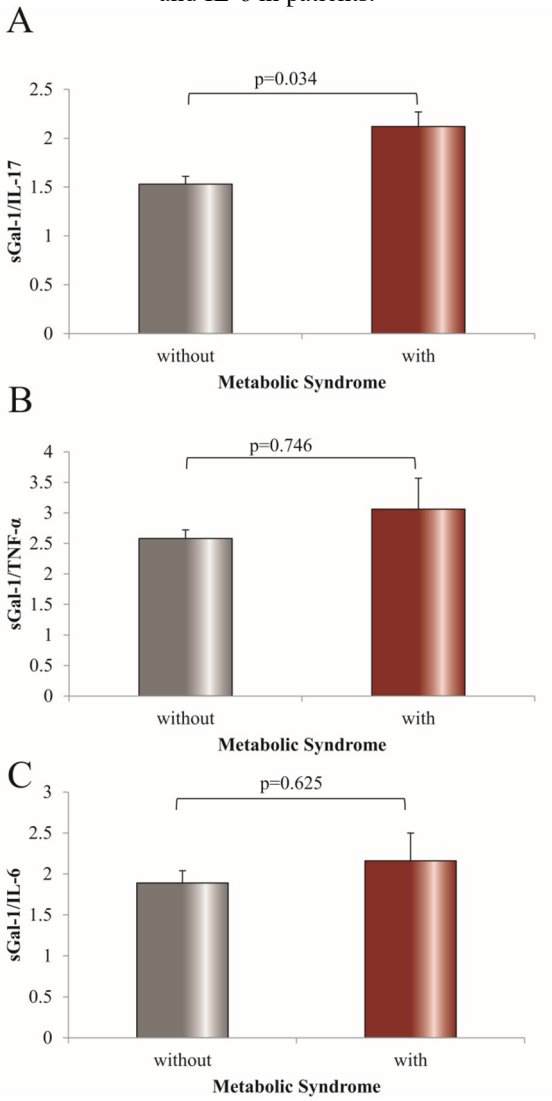
Figure 1. Galectin-3 (Gal-1) levels in patients with ulcerative colitis (UC) with and without metabolic syndrome (MetS).



The figure displays serum (**A**) and fecal (**B**) Gal-1 values. The Student's t-test or the Mann-Whitney U test was utilized to assess statistically significant differences, as appropriate. Values are presented as mean ± standard error of the mean. Note that Gal-1 refers to Galectin-1 in this context.

It is hypothesized that the ratio of counter-regulatory cytokines may serve as a relevant disease indicator. Consequently, we analyzed the interrelationships of the examined biomarkers. Figure 2 presents the relationship between serum levels of Gal-1 and pro-inflammatory cytokines TNF-α, IL-6, and IL-17. There was a significant difference in the ratio of serum Gal-1 to IL-17 (2.12±0.15 vs 1.53±0.08; p=0.034) (Figure 2A). The ratios of Gal-1/TNF-α (3.06±0.51 vs 2.58±0.14; p=0.746) and Gal-1/IL-6 (2.16±0.34 vs 1.89±0.15; p=0.625) were higher in the MetS patient group, albeit not statistically significant (Figures 2B and 2C).

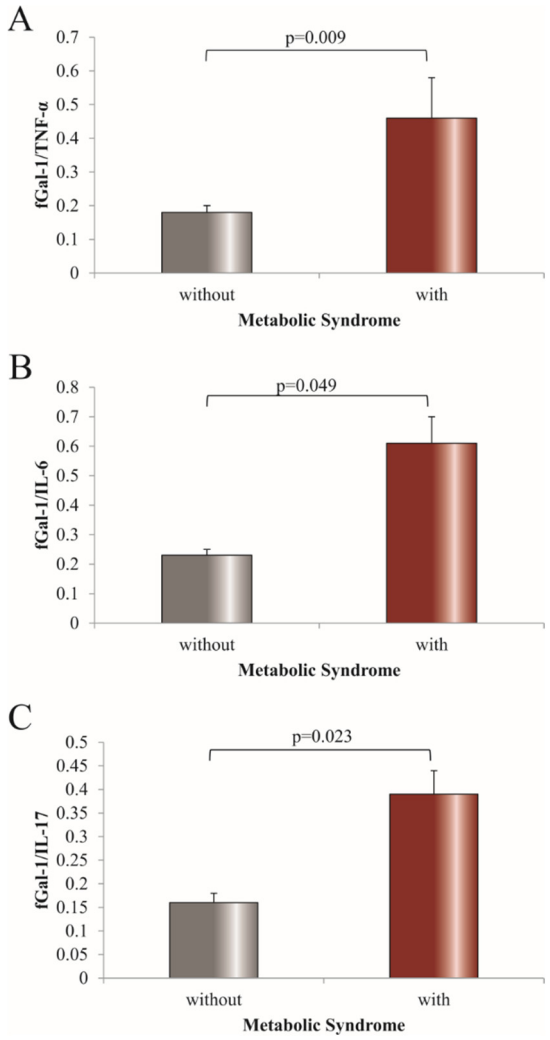
Figure 2. Relationships between serum Gal-1 and pro-inflammatory cytokines TNF-α, IL-17, and IL-6 in patients.



(**A**) Shows the ratio of serum Galectin-1 to IL-17 (sGal-1/IL-17). (**B**) Shows the ratio of serum Galectin-1 to TNF-α (sGal-1/TNF-α). (**C**) Shows the ratio of serum Galectin-1 to IL-6 (sGal-1/IL-6). Statistical significance was tested using the Mann-Whitney U test. Values are presented as mean ± standard error of the mean. Please note: Tumor necrosis factor α (TNF-α), Interleukin-17 (IL-17), Interleukin-6 (IL-6), and Galectin-1 (Gal-1) are the analytes involved.

As demonstrated in Figure 1B, the concentration of Gal-1 was significantly higher in the feces of individuals with UC and MetS. Subsequently, we determined the ratio of Gal-1 to pro-inflammatory cytokines TNF-alpha, IL-6, and IL-17 in patient feces. Figure 3A shows that the ratios of Gal-1 to TNF-α were significantly higher in the patient group with MetS (0.46±0.12 vs 0.18±0.02; p=0.009). Examination of Gal-1/IL-6 in feces (Figure 2B) also revealed significantly higher ratio values (0.61±0.09/0.36 vs 0.23±0.02/0.20; p=0.049) in patients with MetS. Similar to the Gal-1/IL-17 ratio in serum, this ratio was also statistically higher in UC patients' feces (0.39±0.05 vs 0.39±0.05; p= 0.023). These results indicate a dominance of Gal-1 over pro-inflammatory cytokines in both serum and feces of patients with ulcerative colitis and metabolic syndrome.

Figure 3. Association between fecal Galectin-1 (Gal-1) and pro-inflammatory cytokines TNF-α, IL-17, and IL-6 in patients.



Each patient was evaluated separately. (**A**) Illustrates the ratio of fecal Galectin-1 to Tumor Necrosis Factor α (fGal-1/TNF-α). (**B**) Shows the ratio of fecal Galectin-1 to Interleukin-6 (fGal-1/IL-6). (**C**) Depicts the ratio of fecal Galectin-1 to Interleukin-17 (fGal-1/IL-17).

Statistical significance was determined using the Mann-Whitney U test. Results are presented as the mean ± standard error of the mean. Please note: Tumor necrosis factor α (TNF-α), Interleukin-17 (IL-17), Interleukin-6 (IL-6), and Galectin-1 (Gal-1) are the analytes involved.

DISCUSION

In this study, we sought to explore the intersection of Ulcerative Colitis (UC), Metabolic Syndrome (MetS), and their relationship to galectin-1 (Gal-1), a biomarker with proposed immunomodulatory properties. The results of our investigation have revealed several compelling findings that significantly contribute to the existing body of research.

Our study noted elevated levels of creatinine, urea, AST, ALT, triglycerides, and cholesterol in UC patients with MetS compared to those with UC alone, aligning with previous literature and indicating metabolic dysregulation in MetS patients (3, 16). Interestingly, despite these heightened metabolic markers and chronic inflammation typically associated with MetS, the severity of UC was lesser in MetS patients. This is in line with our previous study (16), suggesting a potential interaction between systemic metabolic factors and local gut inflammation that merits further exploration.

Galectin-1, a recently identified peptide primarily released from adipose tissue, has been shown to have anti-inflammatory effects, although its specific function is not precisely defined (17). The aim of our study was to compare the relationship between serum and fecal levels of Gal-1 in relation to metabolic syndrome. Though we found no significant difference in serum concentrations of Gal-1 between UC patients with and without MetS, we observed that patients with MetS had a significantly higher fecal concentration of Gal-1. The reason behind this discrepancy remains unclear, but it might be due to local changes in the gut environment.

Previous studies have reported elevated systemic levels of Gal-1 in metabolic disorders (9). For instance, Acar et al. found significantly higher serum levels of Gal-1 in obese children compared to those of normal weight (18). The level of Gal-1 was significantly increased in the subcutaneous dialysate of patients with type 2 diabetes compared to healthy controls (19). Moreover, the expression of messenger RNA for Gal-1 was found to be increased in the adipocytes of patients with type 2 diabetes (20). In line with these findings, serum Gal-1 was 4.8 times higher in diabetic samples compared to controls (20). These results point to a potential role of Gal-1 in the genesis of metabolic disorders and identify Gal-1 as a new diagnostic marker for these diseases.

In our investigation, we found higher local levels of Gal-1 (Figure 2), and a predominance of Gal-1 over pro-inflammatory cytokines TNF-α, IL-6, and IL-17 (Figures 3 and 4) in patients with metabolic syndrome. Galectin-1 can function both intracellularly and extracellularly following secretion (21). Soluble Gal-1 interacts with fibronectin, integrin, laminin, and Vascular Endothelial Growth Factor Receptor 2

(VEGFR2) receptors, consequently triggering proliferation, adhesion, migration, or angiogenesis (22, 23). The role of Gal-1 in the onset, progression, and resolution of inflammation is well-defined (24). Earlier studies have revealed that Gal-1 inhibits cell growth and induces apoptosis of activated leukocytes (25). Previous work has also shown that Gal-1 directs the acquired immune response towards type 2, concurrently inhibiting the secretion of Interferon-gamma (IFNγ), TNFα, IL-2, and IL-12 while facilitating the production of type 2 cytokine IL-5 (26, 27). Some studies suggest that Gal-1 may inhibit effector T lymphocytes and consequently suppress the potent immune response initiated by pro-inflammatory cytokines (28). The anti-inflammatory properties of Gal-1 have been confirmed in several models of chronic inflammatory and autoimmune diseases, including experimental autoimmune encephalomyelitis, arthritis, uveitis, hepatitis, and diabetes (29, 30). The use of human recombinant galectin-1 induces reduced secretion of type 1 cytokines (TNF-α, IL-1β, IL-12, and IFN-γ) and inflammation suppression in the digestive tract, specifically through the induction of apoptosis in IFN-γ-producing effector T lymphocytes (31). Increased concentration and predominance of the immunosuppressive activity of Gal-1 restrict the local chronic inflammatory response by inhibiting the activity of infiltrating leukocytes and the secretion of inflammation mediators.

A recent study showed that inflammation, accompanied by the production of pro-inflammatory cytokines (IL-1β, TNF, IL-13), intensifies the binding of Gal-1 to enterocytes and subsequent modulation, which triggers a compensatory effect on enterocytes to protect the epithelium and restore intestinal homeostasis (32). Increased concentration of Gal-1 may be the sign of its protective effect on local intestinal mucosa.

CONCLUSION

Our study substantiates the critical role of galectin-1 (Gal-1) in the pathogenesis of metabolic syndrome (MetS) and ulcerative colitis (UC). We noted increased Gal-1 levels, both systemically and locally, among individuals with MetS, suggesting that Gal-1 might exert a protective and immunosuppressive effect in these conditions. This elevation in Gal-1 concentrations could represent a physiological response aimed at mitigating the chronic low-grade inflammation inherent in MetS and UC. This observation underscores the potential of Gal-1 as a novel diagnostic marker and a potential therapeutic target in the management of MetS and UC. Our findings pave the way for further investigation into the role of Gal-1 in these conditions and warrant further studies to elucidate the therapeutic potential of modulating Gal-1 in the management of UC and MetS.

ACKNOWLEDGMENTS

We would like to acknowledge the invaluable contributions and support of all individuals and institutions involved in this research.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding this research on the modulatory role of Galectin-1 in Ulcerative Colitis with comorbid Metabolic Syndrome.

REFERENCES

1. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(1):17-30.
2. Ungaro R, Mehandru S, Allen PB, Peyrin-Biroulet L, Colombel JF. Ulcerative colitis. Lancet. 2017;389(10080):1756-70.
3. Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, et al. The metabolic syndrome. Endocr Rev. 2008;29(7):777-822.
4. Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: a small protein with major functions. Glycobiology. 2006;16(11):137R-57R.
5. Rabinovich GA, Iglesias MM, Modesti NM, Castagna LF, Wolfenstein-Todel C, Riera CM, et al. Activated rat macrophages produce a galectin-1-like protein that induces apoptosis of T cells: biochemical and functional characterization. J Immunol. 1998;160(10):4831-40.
6. Markovic B, Jovanovic I, Volarevic V, Zdravkovic N, Jovanovic M, Marić V, et al. Potential inversely immunomodulatory roles of Galectin-1 and Galectin-3 in ulcerative colitis. Wulfenia. 2016;23:188-205.
7. Farooq W, Farwa U, Khan FR. The metabolic syndrome and inflammation: role of insulin resistance and increased adiposity. Oman Med J. 2015;30(2):100-3.
8. Fryk E, Silva VRR, Jansson PA. Galectin-1 in Obesity and Type 2 Diabetes. Metabolites. 2022;12(10).
9. Mansour AA, Krautter F, Zhi Z, Iqbal AJ, Recio C. The interplay of galectins-1, -3, and -9 in the immune-inflammatory response underlying cardiovascular and metabolic disease. Cardiovascular Diabetology. 2022;21(1):253.
10. Yorulmaz E, Adali G, Yorulmaz H, Ulasoglu C, Tasan G, Tuncer I. Metabolic syndrome frequency in inflammatory bowel diseases. Saudi J Gastroenterol. 2011;17(6):376-82.
11. Rezaianzadeh A, Namayandeh SM, Sadr SM. National Cholesterol Education Program Adult Treatment Panel III Versus International Diabetic Federation Definition of Metabolic Syndrome, Which One is Associated with Diabetes Mellitus and Coronary Artery Disease? Int J Prev Med. 2012;3(8):552-8.
12. Ikeya K, Hanai H, Sugimoto K, Osawa S, Kawasaki S, Iida T, et al. The Ulcerative Colitis Endoscopic Index of Severity More Accurately Reflects Clinical Outcomes and Long-term Prognosis than the Mayo Endoscopic Score. J Crohns Colitis. 2016;10(3):286-95.
13. Pabla BS, Schwartz DA. Assessing Severity of Disease in Patients with Ulcerative Colitis. Gastroenterol Clin North Am. 2020;49(4):671-88.

14. Vespa E, D'Amico F, Sollai M, Allocca M, Furfaro F, Zilli A, et al. Histological Scores in Patients with Inflammatory Bowel Diseases: The State of the Art. J Clin Med. 2022;11(4).
15. Jovanovic M, Gajovic N, Jurisevic M, Markovic B, Marić V, Jovanovic M, et al. Fecal sST2 correlates with disease severity of ulcerative colitis. Vojnosanitetski pregled. 2018;76:26-.
16. Jovanovic M, Simovic Markovic B, Gajovic N, Jurisevic M, Djukic A, Jovanovic I, et al. Metabolic syndrome attenuates ulcerative colitis: Correlation with interleukin-10 and galectin-3 expression. World J Gastroenterol. 2019;25(43):6465-82.
17. Yu X, Qian J, Ding L, Yin S, Zhou L, Zheng S. Galectin-1: A Traditionally Immunosuppressive Protein Displays Context-Dependent Capacities. Int J Mol Sci. 2023;24(7).
18. Acar S, Paketçi A, Küme T, Tuhan H, Gürsoy Çalan Ö, Demir K, et al. Serum galectin-1 levels are positively correlated with body fat and negatively with fasting glucose in obese children. Peptides. 2017;95:51-6.
19. Fryk E, Sundelin JP, Strindberg L, Pereira MJ, Federici M, Marx N, et al. Microdialysis and proteomics of subcutaneous interstitial fluid reveals increased galectin-1 in type 2 diabetes patients. Metabolism. 2016;65(7): 998-1006.
20. Liu X, Feng Q, Chen Y, Zuo J, Gupta N, Chang Y, et al. Proteomics-based identification of differentially-expressed proteins including galectin-1 in the blood plasma of type 2 diabetic patients. J Proteome Res. 2009;8(3):1255-62.
21. Liu FT, Stowell SR. The role of galectins in immunity and infection. Nat Rev Immunol. 2023:1-16.
22. D'Haene N, Sauvage S, Maris C, Adanja I, Le Mercier M, Decaestecker C, et al. VEGFR1 and VEGFR2 involvement in extracellular galectin-1- and galectin-3-induced angiogenesis. PLoS One. 2013;8(6):e67029.
23. Suzuki O, Abe M. Galectin-1-mediated cell adhesion, invasion and cell death in human anaplastic large cell lymphoma: regulatory roles of cell surface glycans. Int J Oncol. 2014;44(5):1433-42.
24. Almkvist J, Karlsson A. Galectins as inflammatory mediators. Glycoconj J. 2002;19(7-9):575-81.
25. Stowell SR, Qian Y, Karmakar S, Koyama NS, Dias-Baruffi M, Leffler H, et al. Differential Roles of Galectin-1 and Galectin-3 in Regulating Leukocyte Viability and Cytokine Secretion1. The Journal of Immunology. 2008;180(5):3091-102.
26. Allione A, Wells V, Forni G, Mallucci L, Novelli F. Beta-galactoside-binding protein (beta GBP) alters the cell cycle, up-regulates expression of the alpha- and beta-chains of the IFN-gamma receptor, and triggers IFN-gamma-mediated apoptosis of activated human T lymphocytes. J Immunol. 1998;161(5):2114-9.
27. Baum LG, Blackall DP, Arias-Magallano S, Nanigian D, Uh SY, Browne JM, et al. Amelioration of graft versus host disease by galectin-1. Clin Immunol. 2003;109(3): 295-307.
28. Sundblad V, Morosi LG, Geffner JR, Rabinovich GA. Galectin-1: A Jack-of-All-Trades in the Resolution of Acute and Chronic Inflammation. The Journal of Immunology. 2017;199(11):3721-30.
29. Offner H, Celnik B, Bringman TS, Casentini-Borocz D, Nedwin GE, Vandenbark AA. Recombinant human beta-galactoside binding lectin suppresses clinical and histological signs of experimental autoimmune encephalomyelitis. J Neuroimmunol. 1990;28(2):177-84.
30. Liu F-T, Rabinovich GA. Galectins: regulators of acute and chronic inflammation. Annals of the New York Academy of Sciences. 2010;1183(1):158-82.
31. Santucci L, Fiorucci S, Rubinstein N, Mencarelli A, Palazzetti B, Federici B, et al. Galectin-1 suppresses experimental colitis in mice. Gastroenterology. 2003;124(5):1381-94.
32. Muglia CI, Gobbi RP, Smaldini P, Delgado ML, Candia M, Zanuzzi C, et al. Inflammation Controls Sensitivity of Human and Mouse Intestinal Epithelial Cells to Galectin-1. J Cell Physiol. 2016;231(7):1575-85.

SERUM LEVELS OF THYROID HORMONES AND THYROID PEROXIDASE ANTIBODIES IN DIFFERENT TYPES OF GLAUCOMA

Katarina Cupic[1,2], Jovana Srejovic[1,2], Suncica Sreckovic[1,2], Nenad Petrovic[1,2], Dusan Todorovic[1,2], Kristina Dugalic[3,4], Rasa Mladenovic[5] and Miroslav Stamenkovic[6,7]

[1]*University of Kragujevac, Faculty of Medical Sciences, Department of Ophthalmology, Serbia*
[2]*Kragujevac University Clinical Center, Clinic of Ophthalmology, Kragujevac, Serbia*
[3]*University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Serbia*
[4]*Kragujevac University Clinical Center, Clinic of Nephrology, Kragujevac, Serbia*
[5]*University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Serbia*
[6]*Zvezdara University Medical Center, Belgrade, Serbia*
[7]*University of Belgrade, Faculty of Special Education and Rehabilitation, Belgrade, Serbia*

Received: 7.5.2024.
Accepted: 31.5.2024.

Corresponding author:

Katarina Cupic

Kragujevac University Clinical Center, Clinic of Ophthalmology, Zmaj Jovina 30, 34000 Kragujevac, Serbia

E-mail: katarinac290@gmail.com

UDK:

Eabr 2026; 27(2):143-147
DOI:10.2478/eabr-2024-0025

ABSTRACT

Aim of this study is to analyze connection between thyroid hormones disbalance and thyroid peroxidase antibodies in serum and different types of glaucoma. This was a hospital-based, retrospective case-control study. Eighty-seven patients were recruited for outdoor department of Clinic of ophthalmology for the routine ophthalmological examination from an endocrinologist, with laboratory analysis of serum levels of thyroid hormones and thyroid peroxidase antibodies test. We specifically included patients with primary open-angle glaucoma and pseudoexfoliation glaucoma, since these two types of glaucoma are highly reported clinically. Based on laboratory analysis, we divided patients into three groups: primary open-angle glaucoma patients with hypothyroidism, pseudoexfoliation glaucoma patients with hypothyroidism and control group that included healthy subjects. In our research, we found that there were discrete differences between glaucoma patients and control subjects in serum levels of free triiodothyronine and free thyroxine. In other words, these differences were insignificant. Patients with primary open-angle glaucoma and pseudoexfoliation glaucoma had increased serum levels of thyroid stimulating hormone, when compared to patients in control group. This study highlights the interaction between thyroid hormones and glaucoma development. Laboratory analysis of glaucoma patients confirmed thyroid peroxidase antibodies levels greater than normal. Control group had physiological range of thyroid peroxidase antibodies. These findings support autoimmune theory of glaucoma development.

Keywords: *Glaucoma, open-angle glaucoma, pseudoexfoliation glaucoma, thyroid hormones, thyroid peroxidase antibodies.*

INTRODUCTION

Glaucoma is one of the most important causes of irreversible blindness in the world due to progressive optic nerve degeneration (1). Based on the state of iridocorneal angle, glaucoma could be classified into two categories: open-angle glaucoma or closed-angle glaucoma. Closed-angle glaucoma has a narrow iridocorneal angle, while open-angle glaucoma is defined by increased resistance in the trabecular meshwork (TM). Both, open-angle glaucoma and closed-angle glaucoma can be primary or secondary. Primary open-angle glaucoma (POAG) is the predominant type of glaucoma (2), while pseudoexfoliation glaucoma (XFG) represents the most important form of secondary glaucoma and is mostly open-angle, but can be also closed-angle glaucoma (3).

POAG is the most highly reported type of glaucoma clinically (4). It is a multifactorial disease in which aging, genetic factors, inflammation and oxidative stress may play a significant role (5).

Pseudoexfoliation syndrome (PEX) is a systemic age-related progressive disorder characterized by abnormal production and aggregation of white extracellular microfibrillar material (6) in multiple organ systems throughout the human body (heart, lungs, kidneys, gallbladder, skin, cerebral meninges, eyes etc.) (7). If PEX deposits accumulate in the anterior segment of the eye, they can obstruct the normal outflow of aqueous humour (AH) causing increased intraocular pressure (IOP) and therefore XFG (8), (9).

PEX may manifest unilaterally or bilaterally. XFG initially develops in one eye before exposed in the other (10). Among the types of glaucoma, XFG has worse prognosis, more damaged visual fiels, a more rapid clinical course, greater optic nerve damage, poorer response to medical treatment and disposes patients to more risk of cataract surgical complications (11).

There are many interactions between thyroid hormones and different diseases. Eyes can also be affected by thyroid gland hormones. This range goes from thyroid-associated orbitopathy (12) to eye changes in hypothyroidism such as conjunctival and periorbital oedema and blepharoptosis (13). This suggests that IOP might be affected by thyroid hormones (14).

However, data regarding connection of thyroid hormones with glaucoma are still poor and inconsistent. Smith's study pointed out that newly diagnosed patients with hypothyroidism had poor outflow of AH, examined with tonography and tonometry (15). After medical treatment of hypothyroidism, AH outflow normalized. Another study didn't find any correlation between IOP and hypothyroidism (16). On the contrary, none of the one hundred patients with hypothyroidism, who had complete ophthalmological examination before and after thyroxine treatment, had been classified as glaucomatous. Lin and others demonstrated a significantly increased risk of open-angle glaucoma development in hypothyroid patients during the 5-year follow-up period, suggesting protective role of levothyroxine (17). Wang and others included 11 studies in their meta-analysis, identifying that hypothyroidism increases risk of POAG development (18). Another study, based on United States population samples, reported lack of connection between hypothyroidism and glaucoma development (19). These contradictory results recommend furthers research of hypothyroidism and glaucoma association.

Thyroid peroxidase is an enzyme that is very important for thyroid hormones' production. Thyroid peroxidase antibodies (TPO-ab) levels increase when immune system falsely attacks and damages thyroid gland cells. These antibodies are found in patients with Hashimoto's thyroiditis and Graves' disease. So far, noone identified significance of TPO-ab in glaucoma disease.

Purpose of this study is to analyze connection between thyroid hormones disbalance and TPO-ab levels in serum with POAG and XFG.

MATERIALS AND METHODS

This is a hospital-based retrospective case-control study. The research was performed from June 2023 until December 2023 at Clinic of ophthalmology, University Clinical Center of Kragujevac. The patients were recruited for outdoor department of Clinic for the routine ophthalmological examination from an endocrinologist, with laboratory analysis of serum levels of thyroid hormones and TPO-ab test.

We analyzed serum levels of thyroid stimulation hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4) and TPO-ab. Based on these results, we divided patients into three groups: POAG patients with hypothyroidism, XFG patients with hypothyroidism and control group that included healthy subjects.

All patients that participated in this study had complete ophthalmological examination: visual acuity test, measured IOP with Goldmann applanation tonometer, biomicroscopic examination of anterior segment, gonioscopy and anterior chamber angle evaluation, as well as fundoscopy. We included patients' gender and age (> 40) as an important data for our study.

POAG patients were defined with presence of retinal nerve fiber layer (RNFL) thinning or defects in visual field followed with IOP levels > 21 mmHg, gonioscopy with the open angle or optic nerve abnormalities (vertical cupping rate > 0.3 cup-disc, asymmetric cupping, peripapillary atrophy etc.) XFG was diagnosed by biomicroscopic examination of characteristic white PEX material over the lens or the iris surface, along with the examination findings counted for POAG. Glaucoma patients, that were included in our study, were using at least one type of anti-glaucoma eye drops.

Blood samples were obtained from the antecubital vein in all patients. The analysis of TSH, fT3, fT4 and TPO-ab levels

was performed after the samples stood for 30 minutes in flat tubes and then centrifuged 1500g for 15 minutes. For the diagnosis of hypothyroidism, we used the laboratory reference range for TSH 0.34-5.60 mlU/L, fT3 2.3-4.1 pg/mL and fT4 0.7-1.9 ng/dL. While evaluating TPO-ab, we checked their normal range which should be < 9.0 IU/ml.

IBM SPSS Statistics ver. 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. For comparing TSH, fT3, fT4 and TPO-ab levels among the groups paired t-test and ANOVA were used ($p<0.05$ and $p<0.001$ were considered as statistically significant.)

RESULTS

The study included 87 patients who were divided into three groups: POAG group (n=31), XFG group (n=22) and control group (n=34). Sixty-eight were females (76.16%) and nineteen males (21.84%). Highly statistically significant difference among sex distribution was recorded in POAG and XFG group ($p>0.001$) (Table 1).

Table 1. Patients' sex distribution

	POAG	XFG	Control
Female	23	25	20
Male	2	3	14
Significance	p<0.001	p<0.001	p<0.05

Mean patients' age in the study was 66.7 ± 6.8 years (median 71, range 42-74 years). The highest mean age was obtained in XFG group (68.4 ± 3.8 years), but without statistically significant difference compared to other groups ($p>0.05$) (Table 2).

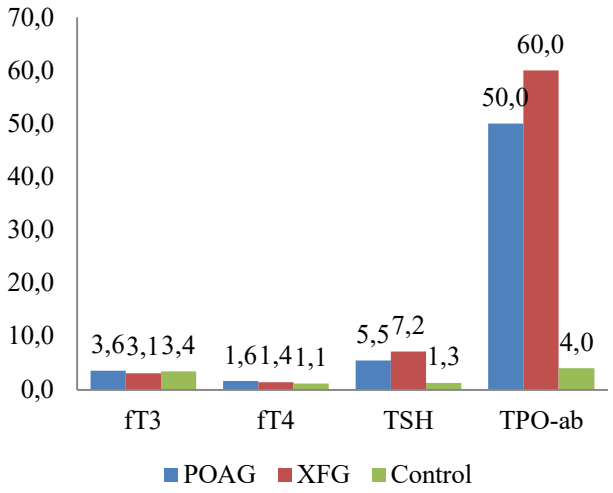
Table 2. Patients' age distribution

Groups	n	Mean	Sd	Range
POAG	31	66.1	4.2	44-72
XFG	22	68.4	3.8	51-74
Control	34	65.7	5.2	42-70

The fT3 and fT4 values were in the range of physiological values in all groups. No statistical significance was seen among the groups in the analysis of fT3 and fT4 values ($p>0.05$). The highest fT3 value was measured in POAG group (3.6 ± 0.3 pg/mL), then in control group (3.4 ± 0.2 pg/mL), and then in XFG group (3.1 ± 0.4 pg/mL). The highest fT4 was also recorded in POAG group (1.6 ± 0.2 ng/dL), then in XFG group (1.4 ± 0.3 ng/dL), then in the control group (1.1 ± 0.3 ng/dL).

Thyroid stimulating hormone was significantly higher in XFG and POAG groups compared to healthy subjects in control group ($p<0.05$). Comparing XFG and POAG groups no statistical significance was notices ($p>0.05$). The highest TSH value was measured in XFG group (7.2 ± 1.4 mlU/L), then in POAG group (5.5 ± 2.5 mlU/L) and then in the control group (1.3 ± 1.1 mlU/L). The same trend was observed in analysis of TPO-ab values. Patients with XFG (60 IU/ml) and POAG (50 IU/ml) had TPO-ab values above the range of physiological values. These results were significantly higher compared to control group (4 IU/ml) ($p<0.05$) (Figure 1).

Figure 1. Values of fT3, fT4, TSH and TPO-ab according to the groups



DISCUSSION

This clinical study demonstrates that there is a statistically significant association between hypothyroidism and glaucoma development. This study is the first of its kind which correlates TPO-ab serum levels and glaucoma development.

Studies that investigate correlation of hypothyroidism and POAG already exist, but there is not enough evidence about possible association between thyroid diseases and XFG (13), (16), (17), (18), (19), (20). Therefore the effect of thyroid hormones' levels in serum on glaucoma development should be more investigated, as well as the potential significant role of TPO-ab.

So far, existing studies include opposed conclusions about the potential connection between thyroid malfunction and glaucoma. Cheng and Perkins reported that among patients with thyroid disfunction, no more than 2% had glaucoma (21). Smith and others found that 23,4% of POAG patients had hypothyroidism (13). They also pointed out a chance that increased IOP could be normalized after treatment of hypothyroidism (15).

It is known that mucopolysaccharides accumulate in dermis and other tissues in patients with hypothyroidism (22). Hyaluronic acid, as a representative mucopolysaccharide and an important part of extracellular matrix, coats the trabecular meshwork, sticking endothelial membranes together (22). Hyaluronic acid accumulates in excessive amount in structures of the eye, creating AH outflow resistance and elevating

IOP in hypothyroidism (23). Stein and others found that AH outflow resistance was decreased in 15% of normal eyes and in 48% of POAG eyes after subconjunctival injection of hyaluronidase (24). They suggested that hyaluronic acid takes an important part in the pathogenesis of POAG.

Many studies reveal that patients with PEX material have increased deposition of hyaluronic acid that coats microfibrils of PEX in lens, zonules, iris and ciliary body (25). Also, there are thyroid receptor isoforms on cells of the trabecular meshwork (22). It has been shown that, by bonding with these receptor isoforms, fT3 reduces hyaluronic acid levels. Furthermore, both disorders, hypothyroidism and XFG, have an undesired harmful effect on the vascular endothelium. Those vascular endothelial cells could have a significant role in the ischemia of glaucomatous optic nerve damage (26).

There are studies that suggest that an abnormal immune response to a sensitizing antigen may induce destruction of retinal ganglion cells and the optic nerve (27), (28). Wax and others discovered autoimmune antibodies against heat shock protein 60 in patients with normal-tension glaucoma (29). Later, other authors detected different antibodies in serum of glaucoma patients such as gamma-enolase (30), glutathione-S-transferaze (31), antiphosphatidylserine (32), neuronspecific-enolase (33) etc. This suggests an autoimmune involvement in glaucoma initiation. To support this autoimmune theory, we examined TPO-ab serum levels.

While analyzing fT3 and fT4 serum levels in our glaucoma patients and control subjects, we found no statistical significance among the groups. On the other hand, TSH levels were higher in XFG and POAG groups compared to healthy subjects, which indicates that patients with hypothyroidism have more risk of glaucoma development. Among glaucoma patients, patients with XFG had more increased TSH levels. This supports previous studies that both, patients with hypothyroidism and patients with PEX material, have increased accumulation of hyaluronic acid which creates elevation of IOP.

TPO-ab in control group was in physiological values. Glaucoma patients had increased TPO-ab levels. It is known that the most common cause of hypothyroidism is an autoimmune response, but our results also support autoimmune theory of glaucoma development. It is possible that TPO-ab can be responsible for both, hypothyroidism and glaucoma initiation.

Our study has several limitations. First, 87 patients were included, so future studies should involve more individuals. Also, our hospital-based retrospective case-control study was performed only from June 2023 until December 2023. Prospective longitudinal studies are needed to assess whether or not hypothyroidism is truly a risk factor for glaucoma initiation and progression of disease, analyzing longer period of time. Our glaucoma patients had increased TPO-ab levels, but their potential pathogenic role in glaucoma development should be more investigated.

CONCLUSION

POAG and XFG have been established to be multifactiorial diseases. They are often the resulf of aging, genetic factors, inflammation and oxidative stress. This study highlights the interaction between thyroid hormones and POAG and XFG development. Patients with hypothyroidism have abnormal accumulation of hyaluronic acid in dermis and other tissues of the human body, as well as in structures of the eye, which leads to increased AH outflow resistance and elevated IOP. Free triiodothyronine reduces hyaluronic acid levels by bonding with thyroid receptor isoforms on TM cells. Previous studies suggest the existence of abnormal immune response which induces the destruction of retinal ganglion cells and the optic nerve in glaucoma. On the other hand, TPO-ab are found in patients with Hashimoto's thyroiditis and Graves' disease. We found that our glaucoma patients had increased TPO-ab values, while healthy subjects in control group had TPO-ab in physiological values. This result supports autoimmune theory of glaucoma development.

ACKNOWLEDGMENTS

None declared.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. Ophthalmology. 2014; 121, 2081-2090. DOI: 10.1016/j.ophtha.2014.05.013.
2. Weinreb RN, Leung CK, Crowston JG, Medeiros FA, Friedman DS, Wiggs JL, et al. Primary open-angle glaucoma. Nat Rev Dis Primers. 2016; 2,160-167. DOI: 10.1038/nrdp.2016.67.
3. Miglior S, Bertuzzi F. Exfoliative glaucoma: new evidence in the pathogenesis and treatment. Prog Brain Res. 2015; 221, 233-241. DOI: 10.1016/bs.pbr.2015.06.007.
4. Yumori JW, Cadogan MP. Primary open-angle glaucoma: clinical update. J Gerontol Nurs. 2011; 37(3), 10-15. DOI: 10.3928/00989134-20110210-01.
5. Janssen SF, Gorgels TGMF, Ramdas WD. The vast complexity of primary open angle glaucoma: disease genes, risks, molecular mechanisms and pathobiology. Progress in Retinal and Eye Research. 2013; 37, 31–67. DOI: 10.1016/j.preteyeres.2013.09.001.
6. Myer C, Abdelrahman L, Banerjee S, Khattri RB, Merritt ME, Junk AK. Aqueous humor metabolite profile of pseudoexfoliation glaucoma is distinctive. Molecular Omics. 2020; 16(5), 425–435. DOI:10.1039/C9MO00192A.
7. Sarenac-Vulovic T, Janicijevic-Petrovic M, Vulovic D, Pavlovic S, Simovic S, Zdravkovic N. Systemic

manifestations of pseudoexfoliation. Ser J Exp Clin Res. 2014; 15(1), 29-32. DOI: 10.2478/SJECR-2014-0004.
8. Rasmussen CA, Kaufman PL. The trabecular meshwork in normal eyes and in exfoliation glaucoma. J Glaucoma. 2014; 23, S15-9. DOI: 10.1097/IJG.0000000000000106.
9. Schlötzer-Schrehardt U. New pathogenetic insights into pseudoexfoliation syndrome/glaucoma. Therapeutically relevant?. 2012; 109 (10), 944-951. DOI: 10.1007/s00347-012-2531-1.
10. Ritch R, Schlotzer-Schrehardt U. Exfoliation syndrome. Survey of Ophthalmology. 2001; 45(4), 265–315. DOI: 10.1016/s0039-6257(00)00196-x.
11. Palko JR, Qi O, Sheybani A. Corneal alterations associated with pseudoexfoliation syndrome and glaucoma: A literature review. J. Ophthalmic Vis.Res. 2017; 12(3), 312–324. DOI: 10.4103/jovr.jovr_28_17.
12. Cockerham KP, Pal C, Jani B, Wolter A, Kennerdell JS. The prevalence and implications of ocular hypertension and glaucoma in thyroid-associated orbitopathy. Ophthalmology. 1997; 104(6), 914-917. DOI: 10.1016/s0161-6420(97)30207-3.
13. Smith KD, Arthurs BP, Saheb N. An association between hypothyroidism and primary open-angle glaucoma. Ophthalmology. 1993; 100(10),1580-1584. DOI: 10.1016/s0161-6420(93)31441-7.
14. Manor RS, Kurz O, Lewitus Z. Intraocular pressure in endocrinological patients with exophthalmos. Ophthalmologica. 1974; 168(4), 241–252. DOI:10.1159/000307046.
15. Smith KD, Tevaarwerk GJ, Allen LH. An ocular dynamic study supporting the hypothesis that hypothyroidism is a treatable cause of secondary open-angle glaucoma. Can J Ophthalmol. 1992; 27(7), 341-344.
16. Karadimas P, Bouzas EA, Topouzis F, Koutras DA, Mastorakos G. Hypothyroidism and glaucoma. A study of 100 hypothyroid patients. Am J Ophthalmol. 2011; 131, 126-128. DOI: 10.1016/s0002-9394(00)00724-8.
17. Lin HC, Kang JH, Jiang YD, Ho JD. Hypothyroidism and the risk of developing open-angle glaucoma: a five-year population based follow-up study. Ophthalmology. 2010; 117, 1960-1966. DOI: 10.1016/j.ophtha.2010.02.005.
18. Wang S, Liu Y, Zheng G. Hypothyroidism as a risk factor for open angle glaucoma: A systematic review and meta-analysis. PloS one. 2017; 12(10), e0186634. DOI: 10.1371/journal.pone.0186634.
19. Kakigi C, Kasuga T, Wang SY, Singh K, Hiratsuka Y, Murakami A, et al. Hypothyroidism and Glaucoma in The United States. PloS one. 2015; 10(7), e0133688. DOI: 10.1371/journal.pone.0133688.
20. Motsko SP, Jones JK. Is there an association between hypothyroidism and open-angle glaucoma in an elderly population? An epidemiologic study. Ophthalmology. 2008; 115(9), 1581-1584. DOI: 10.1016/j.ophtha.2008.01.016.
21. Cheng H, Perkins ES. Thyroid disease and glaucoma. Br J Ophthalmol. 1967; 51(8), 547-553. DOI: 10.1136/bjo.51.8.547.
22. Duncan KG, Jumper MD, Ribeiro RC, et al. Human trabecular meshwork cells as a thyroid hormone target tissue: presence of functional thyroid hormone receptors. Graefes Arch Clin Exp Ophthalmol. 1999; 237(3),231-240. DOI:10.1007/s004170050224.
23. Bahceci UA, Ozdek S, Pehlivanli Z, Yetkin I, Onol M. Changes in intraocular pressure and corneal and retinal nerve fiber layer thicknesses in hypothyroidism. Eur J Ophthalmol. 2005; 15(5),556-561. DOI: 10.5301/EJO.2008.1004.
24. Stein R, Romano A, Treister G, Bartov E. Effect of subconjunctival injection of hyaluronidase on outflow resistance in normal and in open-angle glaucomatous patients. Metab Pediatr Syst Ophthalmol. 1982; 6, 169-174.
25. Gartaganis SP, Georgakopoulos CD, Exarchou AM, et al. Increased aqueous humor basic fibroblast growth factor and hyaluronan levels in relation to the exfoliation syndrome and exfoliative glaucoma. Acta Ophthalmol Scand. 2001; 79(6),572-575.
26. Gao C, Li T, Liu J, Guo Q, Tian L. Endothelial functioning and hemodynamic parameters in rats with subclinical hypothyroid and the effects of thyroxine replacement. PloS one. 2015; 10(7), e0131776. DOI: 10.1371/journal.pone.0131776.
27. Wax MB. Is there a role for the immune system in glaucomatous optic neuropathy? Curr Opin Ophthalmol. 2000; 11(2),145-150. DOI: 10.1097/00055735-200004000-00014.
28. Cartwright MJ, Grajewski AL, Friedberg ML, Anderson DR, Richards DW. Immune-related disease and normal-tension glaucoma. A case–control study. Arch Ophthalmol. 1992; 110(4),500-502. DOI: 10.1001/archopht.1992. 01080160078035.
29. Wax MB, Tezel G, Saito I, et al. Anti-Ro/SS-A positivity and heat shock antibodies in patients with normal-pressure glaucoma. Am I Ophthalmol. 1998; 125(2),145-157. DOI: 10.1016/s0002-9394(99)80084-1.
30. Maruyama I, Ohguro H, Ikeda Y. Retinal ganglion cells recognized by serum autoantibody against gamma-enolase found in glaucoma patients. Invest Ophthalmol Vis Sci. 2000; 41(7),1657-1665.
31. Yang J, Tezel G, Patil RV, Romano C, Wax MB. Serum autoantibody against glutathione S-transferase in patients with glaucoma. Invest Ophthalmol Vis Sci. 2001; 42(6),1273-1276.
32. Kremmer S, Kreuzfelder E, Klein R, Bontke N, Henneberg-Quester KB, Steuhl KP, GrosseWilde H. Antiphosphatidylserine antibodies are elevated in normal-tension glaucoma.Clin Exp Immunol. 2001; 125(2),211-215.DOI:10.1046/j.1365-2249.2001.01578.x.
33. Ikeda Y, Maruyama I, Nakazawa M, Ohguro H. Clinical significance of serum antibody against neuron-specific enolase in glaucoma patients. Jpn J Ophthalmol. 2002; 46(1),13-17. DOI: 10.1016/s0021-5155(01)00455-5.

DISABILITY AND DEPRESSIVENESS ARE RELATED TO IL-18 SERUM LEVELS IN THE REMISSION OF MULTIPLE SCLEROSIS

Katarina Vesic[1], Aleksandar Arsenijevic[2], Jelena Milovanovic[2], Nataša R. Mijailović[3] and Aleksandar Gavrilovic[1]

[1] *University of Kragujevac, Faculty of Medical Sciences, Department of Neurology, Kragujevac, Serbia*
[2] *University of Kragujevac, Faculty of Medical Sciences, Center for Molecular Medicine and Stem Cell Research, Kragujevac, Serbia*
[3] *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia*

Received: 25.10.2023.
Accepted: 08.02.2024.

Corresponding author:

Katarina Vesic, MD, Ph.D.

Department of Neurology, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia

Phone: +381 607129294

E-mail: stojanovick@yahoo.com

UDK:

Eabr 2026; 27(2):149-158
DOI: 10.2478/eabr-2024-0002

ABSTRACT

Depression and fatigue are common comorbidities in multiple sclerosis patients, and multiple sclerosis, depression, and fatigue are all associated with dysregulated cytokine production. The serum levels of interleukin-18 , C-reactive protein , and uric acid were measured in 48 multiple sclerosis patients in the relapse before and 2 months after corticosteroid treatment, 50 multiple sclerosis remitting patients, and 33 healthy subjects. The severity of depressive symptomatology, fatigue, and disability was assessed using the Beck's Depression Inventory , the Fatigue Severity Scale , and the Expanded Disability Status Scale , respectively. We also investigated the possible correlation of these biomarkers with clinical symptomatology scores. Multiple sclerosis patients had significantly higher levels of Interleukin-18 compared to healthy control subjects (468.96 ± 840.77 vs. 405.20 ± 146.16 pg/ml, p = 0.017), while there were no differences between groups of patients. We found a moderate positive correlation between levels of Interleukin-18 and C-reactive protein in the relapse phase (p = 0.012, r = 0.305) and a strong negative correlation between levels of Interleukin-18 and uric acid in the remission phase (p = 0.014, r = -0.551). We found a strong positive correlation between concentrations of Interleukin-18 and Beck's Depression Inventory score (p = 0.018, r = 0.535) and a moderate positive correlation with Expanded Disability Status Scale score (p = 0.047, r = 0.462) in the stabilization of multiple sclerosis. Our results may suggest that Interleukin-18 is involved in inflammation in the acute phase and regresses in the stabilization phase, but also in the psychopathology that often occurs after multiple sclerosis.

Keywords: *Multiple sclerosis, interleukin-18, C-reactive protein, uric acid, depression.*

INTRODUCTION

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS), mediated by immune and inflammatory mechanisms (1). The immune response is involved in its pathogenesis, and an imbalance between pro- and anti-inflammatory cytokines has been demonstrated in numerous studies showing a correlation with changes in MS disease activity (2, 3, 4, 5, 6). All these perturbations could be useful for further improvement of clinical practice.

In addition to the various functions of cells of the immune system in the periphery, cells of the nervous system, microglia, and astrocytes are also important sources of cytokines (7). By acting on specific structures in the brain, proinflammatory cytokines can cause behavioural changes (8, 9). The main symptoms of this inflammation-induced behaviour are fatigue, anhedonia, anorexia, depression, and increased pain sensitivity (10, 11, 12). One of the proposed theories is that the behavioural changes represent a variant of "inflammation-induced sickness behaviour", which is an adaptive defence mechanism against infection (13). The onset of sickness behaviour is associated with infiltration of inflammatory cells in the brain and increased mitochondrial ribonucleic acid (mRNA) expression of interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and prostaglandin E2 in brain tissue, whereas anti-inflammatory treatment with dexamethasone, interleukin-1 (IL-1) receptor antagonist (IL-1Ra), and indomethacin ameliorates behavioural symptoms (14, 15). The production of TNF-α in acute inflammation is always followed by the production of IL-1β (16).

The blockade of IL-1 in experimental autoimmune encephalomyelitis (EAE) has a protective effect, whereas interferon-beta (IFN-β) induces the expression of IL-1Ra in microglia, increasing the concentration of this mediator in serum (17). Serum levels of pro-inflammatory cytokines are certainly increased in relapsing MS (18). In this context, the relationship between fatigue, depression, and inflammatory cytokines has also been demonstrated in various autoimmune diseases (19, 20). Further, proinflammatory cytokines play an important role in the genesis of MS-related depression and fatigue (21, 22). An increase in the production of cytokines such as TNF-α, interferon-gamma (IFN-γ), and IL-1β has been demonstrated in depressed MS patients (23). Depression and fatigue are symptoms that are strongly correlated, and it has been shown that successful treatment of depression can in turn decrease fatigue (24).

In addition to the aforementioned cytokine IL-1β, cytokine family 1 also includes IL-18, whose active forms, as in the case of IL-1, are released by the action of caspase 1, which is activated by the nucleotide-binding oligomerization domain-, leucine-rich repeat, and pyrin domain-containing 3 (NLRP3) inflammasome (25). IL-18 is a pleiotropic pro-inflammatory cytokine involved in the regulation of innate and adaptive immune responses (26). It exists in an inactive precursor form (proIL-18) and converts to an active form in the presence of caspase-1 in response to inflammatory and infectious stimuli (27, 28). Formerly called interferon-gamma-inducing factor, it causes the production of IFN-γ in T cells and natural killer (NK) cells but can also be produced by various cell types, including monocytes/macrophages, dendritic cells, B cells, and other antigen-presenting cells, as well as astrocytes and microglia (29, 30, 31). Dysregulation of IL-18 production was observed in various autoimmune diseases such as bowel disease, rheumatoid arthritis, systemic juvenile idiopathic arthritis, and lupus erythematosus, suggesting its involvement in inflammatory cascades (32, 33, 34, 35).

Recently, a strong correlation between serum levels of IL-18 and C-reactive protein (CRP) was found in patients with severe Coronavirus infection (36). Further, uric acid (UA) has been extensively studied as an alarmin in MS, and it is known to activate the NLRP3 inflammasome and promote inflammation through maturation and release of the pro-inflammatory cytokines IL-1β and IL-18 (37, 38). Decreased concentrations of UA have been recorded in patients with MS compared to healthy individuals, as well as in relapses compared to concentrations in the remission phase of MS (39). All this suggests that these cascades involving the effects of UA and CRP on IL-18 secretion should be further explored in MS.

The similarities between the IL-1 and IL-18 systems suggest the possibility that, like IL-1β, IL-18 may also be a mediator of the sickness behaviour symptomatology (12, 40). An increased concentration of IL-18 has been confirmed in the serum and cerebrospinal fluid (CSF) of patients with MS, but there are no data on the relationship between this cytokine and fatigue and depressive symptomatology (41).

In this study, we aimed to measure and compare serum levels of IL-18 in patients with MS at different stages, in patients with a stable remitting form, in acute relapses before and after 2 months of treatment with pulse corticosteroid therapy, and in healthy subjects who served as controls. We sought to explore more complex interactions and the presence of a correlation between the levels of IL-18 and CRP, as a peripheral biomarker of inflammation, and UA, as an alarmin, and also, for the first time in this context, its association with clinical scores representing the severity of fatigue and depressive symptomatology.

PATIENTS AND METHODS

Subjects

The study included patients with clinically confirmed MS diagnosis according to the revised McDonald's criteria and matched healthy controls recruited during blood donation at the Blood and Blood Products Service of the University Clinical Centre Kragujevac (42). In addition, the MS patients were divided into two groups: MS patients in the acute relapse phase (MS relapse group) and MS patients in the remitting phase (MS remitting group). An exacerbation of MS or relapse was defined as the appearance of new symptoms that lasted longer than 24 hours and occurred in the absence of

fever and infection, or an exacerbation of existing symptoms after 30 days of a stable phase of the disease (43).

Patients in the MS relapse group were treatment-naïve regarding immunomodulatory therapy. Patients in the relapse phase were referred to the neurology unit because of the current exacerbation of the disease and indicated treatment with pulse corticosteroid therapy. In this group of patients, laboratory measurements and clinical assessments were performed before the initiation of treatment for acute relapse and eight weeks after the use of pulse corticosteroid therapy in the stable phase of the disease. In the remitting MS patients, these procedures were performed at scheduled three-month visits. All patients in the remitting group were treated with IFN-β-1a administered subcutaneously three times weekly.

Exclusion criteria were acute or chronic medical conditions, acute infections, recent surgical procedures, alcohol or drug abuse, any disease that is immune- or redox-mediated, allergies, or autoimmune disorders. The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation and the presented protocol of this study was approved by the Ethics Committee of the University Clinical Centre Kragujevac. Voluntary written informed consent was obtained from each participant prior to enrolment in the study.

Laboratory measurements

Blood samples for the measurement of serum levels of CRP and UA and for the determination of serum cytokine concentrations were obtained in the morning and additionally before the use of corticosteroid therapy in MS recurrent patients. Serum cytokine levels were measured in patients and healthy volunteers using conventional enzyme-linked immunosorbent assay (ELISA) kits (R & D Systems Minneapolis, MN for IL-18 according to the manufacturer's instructions). Immunological measurements were performed at the Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac.

Clinical assessment

Auto- and heteroanamneses were performed to obtain sociodemographic and clinical data. Depressive symptomatology was assessed with the Beck Depression Inventory (BDI), which is recommended by the American Academy of Neurology for screening depressive symptoms in MS patients (44). The 21-item BDI includes somatic and cognitive-affective symptoms, and each item is graded on a 0 to 4 scale reflecting the patient's feelings over the past two weeks. The degree of severity of depression symptoms was quantified as follows: normal (range 0-10), mild mood disturbance (range 11-16), borderline clinical depression (range 17-20), moderate depression (range 21-30), severe depression (range 31-40), and extreme depression (over 40).

The occurrence of fatigue was assessed with a self-report questionnaire - Fatigue Severity Scale (FSS) (45). The scale consists of nine items on a seven-point scale. The presence of mild fatigue is indicated by an average score greater than 3 points, and as disabling fatigue by an average FSS score greater than 4.

The Expanded Disability Status Scale (EDSS) was used to assess the degree of neurological disability (46). The EDSS consists of eight functional domains: visual (optic), brainstem, pyramidal, cerebellar, sensory, bowel and bladder, cerebral function, and ambulation. The EDSS score ranges from 0 (normal neurologic examination) to 10 (death due to MS), in half-point increments, to indicate a mild, moderate or severe disability.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 software (IBM Corp. Armonk, NY, USA). Numerical values are expressed as mean and standard deviation (SD). Normal distribution of the data was tested with the *Shapiro-Wilk test* and the *Kolmogorov-Smirnov test*. A paired-sample test and the *Wilcoxon test* were used to compare the clinical parameters in the same group before and after therapy. For nonparametric variables, the *Mann-Whitney test* was used to detect mean differences between two groups of patients. A *Kruskal-Wallis test* was used to assess differences in IL-18 serum levels between three groups (MS relapse, MS remitting, and HC group). We examined the correlations between IL-18 serum levels and CRP and UA serum levels and the correlations between IL-18 serum levels and EDSS, BDI, and FSS scores using *Pearson's correlation* for parametric or *Spearman's* correlation for nonparametric data. To determine the best prediction of explanatory variables such as sex, age, and serum levels of UA and IL-18 on illness onset, a binary logistic regression analysis was performed. A p-value of 0.05 was considered statistically significant.

RESULTS

Sociodemographic data are shown in Table 1. The study included 133 participants, MS patients (n = 98) and healthy controls (n = 35). Of the MS population, 48 patients or 48.9% were in an acute relapse (MS relapse group) and 50 patients or 51.1% were in a stable phase of the disease and receiving immunomodulatory treatment (MS remitting group). No differences were found between the groups in terms of gender and age. The mean disease duration was significantly longer in the MS remitting group (6.19 ± 7.11 *vs*. 8.64 ± 4.46; p = 0.01), while the relapse rate was higher in the MS relapse group (2.42 ± 1.22 *vs*. 1.24 ± 1.39; p = 0.05).

Table 1. Sociodemographic characteristics of the study population

variables	MS relapse group (*n* =48)	MS remitting group (*n* = 50)	control group (*n* = 35)	*p* - value
age (years)	41.7 ± 9.13	38.6 ± 7.88	38.6 ± 8.73	0.14*
sex (female / male)	30 / 18	35 / 15	22 / 13	0.20*
mean duration of illness (years)	6.19 (1-34)	8.64 (2-18)	NA	**0.01****
relapse rate	2.42 (0-5)	1.24 (0-6)	NA	**0.05****

Abbreviations: MS - multiple sclerosis; NA - not applicable.

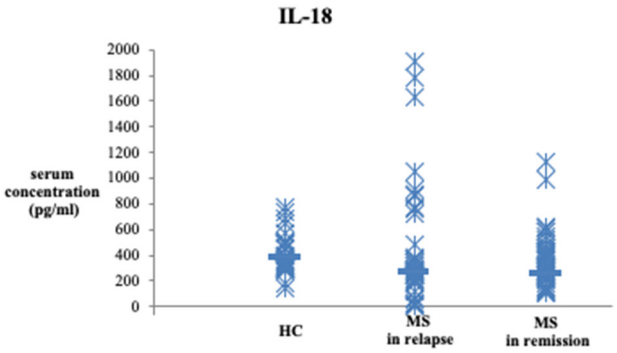
* *The Kruskal-Wallis test* was used to assess the differences between the three groups.

** *The Mann-Whitney test* was performed to compare means in the two groups.

Data are presented as means and standard deviations, $p < 0.05$ considered as significant and those values bolded.

We analysed serum concentrations of IL-18 and observed statistically significant differences between the MS group and healthy controls (468.96 ± 840.77 *vs.* 405.20 ± 146.16 pg/ml, $p = 0.017$). Further, the MS group was divided into the MS relapse and MS remitting groups and the data were compared between all three groups. We found a statistically significant difference in the mean values of IL-18 concentration between MS relapse, MS remitting and the control group (578.41 ± 1160.70 *vs.* 357.195 ± 206.75 *vs.* 405.20 ± 146.16 pg/ml, $p = 0.040$) (presented in Figure 1).

Figure 1. Serum levels of IL-18.

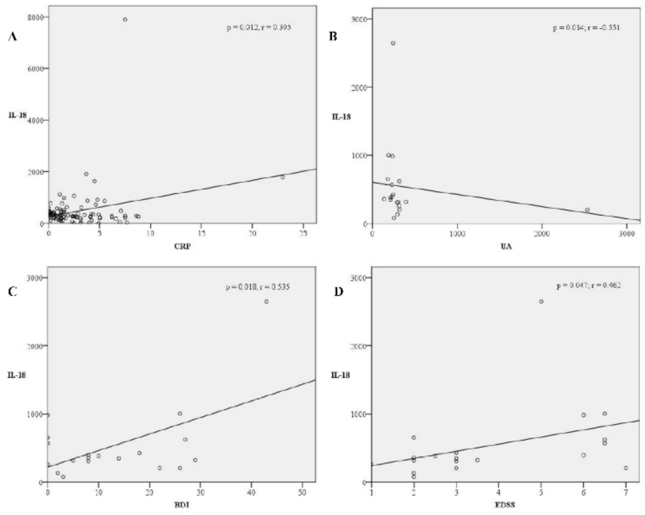


Serum levels of IL-18 were determined by ELISA in healthy control subjects (n = 35), MS relapse patients (n = 48) and MS remitting patients (n = 50). Data are presented as mean ± SD. Statistical significance was tested using the Kruskal-Wallis test. Serum levels of IL-18 were significantly higher in MS relapse and MS remitting patients compared to the group of healthy control subjects (578.41 ± 1160.70 vs. 357.195 ± 206.75 vs. 405.20 ± 146.16 pg/ml, p = 0.040).

In addition, a comparison of serum concentrations of IL-18 in the MS group in relapse and remission revealed no statistically significant differences between these patient groups (578.41 ± 1160.70 *vs.* 357.192 ± 206.75 pg/ml, $p = 0.597$). We also analysed serum levels of IL-18 in the 19 patients in the MS relapse group before and after treatment with pulse corticosteroid therapy and no statistically significant differences were found ($p = 0.658$).

We found a significant, moderate positive correlation between IL-18 and serum levels of CRP in relapse ($p = 0.012$, r = 0.305) (Figure 2A). In remission, a significantly strong negative correlation was observed between IL-18 and serum concentrations of UA ($p = 0.014$, r = -0.551) (Figure 2B). Clinical characteristics (EDSS, FSS and BDI scores) of the sample were previously presented in Vesic et al. (2020) (39). Regarding behavioural changes, we found a statistically significant, strong positive correlation between serum concentrations of IL-18 and BDI score ($p = 0.018$, r = 0.535) (Figure 2C) and a moderate positive correlation with EDSS score ($p = 0.047$, r = 0.462) (Figure 2D) in the stabilization of MS. We found no significant correlation between IL-18 and FSS score.

Figure 2 Legend. Correlations between serum levels of IL-18 and CRP and UA, and BDI and EDSS clinical scores. A significant, moderate positive correlation was observed between IL-18 and serum levels of CRP ($p = 0.012$, $r = 0.305$) in acute relapse (A). In stabilization, after eight weeks of corticosteroid therapy, a significant strong negative correlation was observed between IL-18 and serum concentrations of UA ($p = 0.014$, $r = -0.551$) (B), a strong positive correlation was observed between IL-18 and BDI score ($p = 0.018$, $r = 0.535$) (C), and a moderate positive correlation was observed with EDSS score ($p = 0.047$, $r = 0.462$) (D).



Abbreviations: IL - interleukin; CRP - C-reactive protein; EDSS - Expanded Disability Status Scale; UA - uric acid, BDI - Beck's Depression Inventory.

Complementary binary logistic regression analysis was performed using available data on sex, age, serum IL-18 and UA levels to determine the best predictor of illness onset (Table 2). The results showed that only serum levels of UA influenced illness onset ($p = 0.000$), while sex ($p = 0.950$), age ($p = 0.663$), and serum levels of IL-18 ($p = 0.884$) were not significantly associated with illness onset.

Table 2. Results of binary logistic regression analysis with illness onset as the dependent variable, and sex, age, serum UA and IL-18 levels as explanatory variables.

category	explanatory variables	Wald	df	*p* - value	Odds ratio	Lower and upper 95% CI
illness onset	sex	0.004	1	0.950	0.371	2.878
	age	0.190	1	0.663	0.960	1.066
	UA	15.692	1	0.000	0.979	0.993
	IL-18	0.021	1	0.884	0.999	1.001

Abbreviations: UA - uric acid; IL - interleukin.

DISCUSSION

The results of our study confirmed that the levels of IL-18 were significantly higher in patients with MS compared to the healthy control subjects. There were no significant differences in serum levels of this cytokine in MS relapse compared to the MS remitting group of patients. In addition, a slight but nonsignificant decrease in serum levels was observed after corticosteroid therapy. All these data could imply that IL-18 cannot be considered as a state marker but rather as a trait marker in MS. In the periphery, a positive correlation was found between CRP and IL-18 in the relapse phase and a negative one between UA and IL-18 in a remission phase after acute relapse. These results may suggest that IL-18 is involved in inflammation in the acute phase and regresses in the stabilization phase, which is associated with

increased behavioural symptoms such as fatigue and depression.

Evidence is accumulating that IL-18 is involved in the pathogenesis of MS and other autoimmune diseases (47). In 1998, *Jander and Stoll* observed increased levels of IL-18 mRNA in the spinal cord of rats suffering from the acute phase of EAE (48). In EAE, IL18-/- mice have been shown not to develop the disease (49). In recent years, several studies have presented conflicting results regarding serum levels of IL-18 in MS patients. In the search for genetic factors associated with predisposition to MS, it has been suggested that the IL-18 gene polymorphism increases susceptibility to MS (42, 50). Previously, increased expression of IL-18 was found in demyelinating brain lesions of MS patients, and antibodies against IL-18 were shown to prevent autoimmune encephalomyelitis (51, 52). Similar to our results, increased serum levels of IL-18 were observed in MS patients compared to the control group (53, 54). In addition, *Huang* et al. reported that the expression of IL-18 in serum and the level of IL-18 mRNA in peripheral blood mononuclear cells were significantly increased in patients with MS (55).

We also analysed the serum levels of IL-18 at different stages of MS and there were no significant differences in serum levels of this cytokine in MS relapse compared to the MS remitting group of patients. The highest levels of IL-18 were detected in the MS relapse patient group. The results of *Losy* and colleagues emphasized significantly higher concentrations of IL-18 in patients with gadolinium-enhancing lesions on magnetic resonance imaging compared with patients without active lesions. This fact suggests the involvement of IL-18 in the acute exacerbation and active stage of MS (56). In contrast, *Trenova* et al. examined serum levels of IL-18 in MS-drug-naïve patients and in the stable phase of the disease as well as in healthy control subjects, and they found no significant differences of this cytokine between the groups, indicating that special attention should be paid to the applied therapy (57). Thus, we observed the lowest serum levels of IL-18 in MS patients with long stable phase of MS treated with IFN-β-1a compared to healthy control subjects. Previously, it was reported that the levels of IL-18 decreased significantly during therapy with glatiramer acetate in MS patients (56). In addition, we analysed sera IL-18 levels before, and after corticosteroids treatment in MS relapse patients. Corticosteroids regulate NLRP3-inflammasome activation at both the transcriptional level via blocking NF-κB signal pathway and processing level via suppressing the reactive oxidative species generation and IL-18 levels (58, 59). In our study, a slight but non-significant decrease in serum levels was observed after two months of corticosteroid therapy. A possible reason is a small sample size that only 19 patients had a follow-up after treatment of corticosteroids. In current literature, a limited number of studies have been discussed about IL-18 sera levels in different stages of MS. The relationship of the IL-18, MS phenotype and corticosteroids treatments need to be investigated in the future on a bigger sample size.

To further explore the inflammatory milieu, IL-18 was placed in the context of interactions with CRP and UA. In a previous study, our research group observed significantly higher CRP levels as an inflammatory marker of acute exacerbation compared between MS remitting and relapsing patients and significantly lower UA levels in the serum of MS relapsing patients compared to MS remitting patient groups and healthy control subjects (39). The additional results presented from the same sample showed a significant, moderate positive correlation between CRP and IL-18 serum levels in acute relapse and a significant strong negative correlation between UA and IL-18 levels after treatment of acute relapse with pulse corticosteroid therapy during stabilization of the illness. IL-18 seems to interfere with CRP potentiating inflammation while downregulating UA levels in the acute phase of the illness. Later, after treatment with corticosteroids, it plays a lesser role in the phase of attenuated inflammation (lower CRP and higher UA serum levels), but may be more important in regulating behavioural symptoms such as fatigue and depression.

In present study, we revealed a moderate positive correlation between sera IL-18 levels and EDSS score in stable phase of disease. The findings of Soares et al. emphasized that a constitutive activation of NLRP3 inflammasome, and the consequent increased IL-1β and IL-18 production, represents a risk factor for both, the development of MS and the progression to higher EDSS score and severe forms of the disease. On the other hand, low IL-18 production and/or NLRC4 activation resulted beneficial for MS patients (60).

IL-18-deficient mice exhibited hippocampal abnormalities which resulted in impaired learning and memory, and depressive-like behaviour, but also stress-induced hyperactivity mediated by neuroinflammation and attenuated neurogenesis (61, 62). In addition, administration of IL-18 to the amygdala, but not to the hippocampus, resulted in severe depression-like behaviours (63). *Haastrup* et al. indicated that the genetic tendency toward higher IL-18 production may increase susceptibility to depression in response to stressful life events (64). Several clinical studies reported increased serum levels of IL-18 in patients with depression compared to healthy controls and demonstrated impaired brain activity (65, 66, 67, 68). Our results showed a statistically significant, strong positive correlation between serum concentrations of IL-18 and BDI score in the remission phase. Previous studies observed relationship between depressive symptoms and IL-18 in other inflammatory diseases. Zao et al. showed that IL-18 levels positive correlated with BDI scores in depressed patients with chronic obstructive pulmonary disease (69). Recent studies indicated that increased IL-18 in the brain causes depression-like behaviors by promoting the IL-18 receptor/NKCC1 signaling pathway and that this cytokine is a biomarker for post-stroke depression. The blockage of endogenous IL-18 by IL-18 binding protein, a specific antagonist of IL-18, repressed depressive phenotypes in mice (63). Potential mechanisms through cytokines mediate depression like behavior are various. Proinflammatory cytokines directly stimulate the hypothalamic-pituitary-adrenal axis and

can mitigate serotonin synthesis by inducing the enzyme indoleamine 2, 3 dioxygenase, which catabolizes tryptophan into kynurenine (70, 71). In homeostatic concentrations, IL-18 support neurons and enhance long-term potentiation, but elevated IL-18 and other proinflammatory cytokines may promote the dysregulation of several neurotransmitter pathways, including the dopaminergic, serotoninergic, noradrenergic, and glutamatergic systems (71, 72).

This was clinically confirmed by the fact that the nonsteroidal anti-inflammatory drug ketoprofen decreased IL-18 levels in patients with depression when used as adjunctive treatment and also improved clinical outcomes (73). Future strategies must consider that higher serum UA levels may be responsible for chronic activation of the NLRP3 inflammasome, persistent activation of NF-κB, and elevated levels of pro-inflammatory cytokines, including IL-18, but also in MS related depression (38).

These conflicting results across studies might be a consequence of sample heterogeneity and measurement in different phases of illness. This study was limited in terms of comparative resources (lacked laboratory data from control subjects). In addition, not all patients had follow-up after treatment with corticosteroids ($n = 19$). Data on metabolic parameters and other additional clinical and sociodemographic variables must also be considered in the future.

CONCLUSION

The results of the present study show that the levels of the proinflammatory cytokine IL-18 are increased in MS patients compared with healthy subjects. The positive correlation of IL-18 with serum CRP in the relapse phase and the negative correlation with serum levels of UA in the remission phase suggests its inflammatory role in the active phase of the disease and a regressive one in the stabilization phase of the disease course. The positive correlation of IL-18 with depression and disability scale scores confirms its role as a contributor to depressiveness and disability in MS patients. MS-related depression and fatigue are common and challenging symptoms of MS, which have a significant impact on patients' quality of life. The cytokine pattern in MS patients varies according to disease stage, necessitating an expansion of research on cytokines as potential biomarkers of disease onset, progression, assessment of treatment efficacy and response to treatment.

ETHIC APPROVAL

The study was conducted in accordance with the ethical standards of the committee responsible for human experimentation (institutional) and the Helsinki Declaration of 1975, as revised in 2013. Voluntary written and informed consent was obtained from each participant prior to enrolment in the study. The protocol of the study was approved by the local ethic committee.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This work was supported by the Faculty of Medical Sciences, University of Kragujevac, No. JP 03/16.

ACKNOWLEDGMENTS

Prof. Miodrag L. Lukic and Prof. Nebojsa N. Arsenijevic contributed significantly to initiating this research project and shedding light on possible aspects of the IL-18 special role. Prof. Marija Milovanovic contributed significantly in the realization of the project and immunological measurement. Prof Milica Borovcanin contributed significantly to the concept and manuscript structure and gave some new insights through her specific competencies

We thank Bojana Mircetic for language editing.

REFERENCES

1. Sospedra M and Martin M. Immunology of multiple sclerosis. Annals Review Immunology 2005; 23: 683-747.
2. Graber JJ, Ford D, Zhan M, Francis G, Panitch H and Dhib-Jalbut S. Cytokine changes during interferon-beta therapy in multiple sclerosis: correlation with interferon dose and MRI response. J Neuroimmunol 2007; 185: 168-174.
3. Musette P, Benveniste O, Lim A, Bequet D, Kowilsky P, Dormont D et al. The pattern of production of cytokine mRNAs is markedly altered at the onset of multiple sclerosis. Res Immunol 1996; 147: 435-441.
4. Monteyne P, Van Antwerpen MP, Sindic CJ. Expression of costimulatory molecules and cytokines in CSF and peripheral blood mononuclear cells from multiple sclerosis patients. Acta Neurol Belg 1999; 99: 11-20.
5. Trenova AG, Manova MG, Kostadinova II, Hrsitova DR, Vasileva TV and Zahariev ZI: Clinical and laboratory study of pro-inflammatory and anti-inflammatory cytokines in women with multiple sclerosis. Folia Med 2011; 53: 29-35.
6. Kallaur AP, Oliveira SR, Colado Simão AN, Delicato de Almeida ER, Kaminami Morimoto H, Lopes J et al. Cytokine profile in relapsing-remitting multiple sclerosis patients and the association between progression and activity of the disease. Molecular Medicine Reports 2013; 7: 1010-1020.
7. Bradl M. Immune control of the brain. Springer Semin Immunopathol 1996; 18 (1): 35-49.
8. Dantzer R, Heijnen CJ, Kavelaars A, Laye S, Capuron L. The neuroimmune basis of fatigue. Trends Neurosci 2014; 37: 39-46.
9. Sha Q, Madaj Z, Keaton S, Escobar Galvis ML, Smart L, Krzyzanowski S et al. Cytokines and tryptophan

metabolites can predict depressive symptoms in pregnancy. Transl Psychiatry 2022; 12 (1): 35.
10. Clark IA, Budd AC, Alleva LM. Sickness behaviour pushed too far-the basis of the syndrome seen in severe protozoal, bacterial and viral disease and posttrauma. Malar J 2008; 7: 208-215.
11. Bluthe RM, Walter V, Parnet P, Layé S, Lestage J, Verrier D et al. Lipopolysacharide induced sickness behaviour in rats by a vagal mediated mechanism. Compt Rend l'Acad Sci III 1994: 317: 499-503.
12. Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelly KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008; 9: 46-56.
13. Lasselin J. Back to the future of psychoneuroimmunology: Studying inflammation-induced sickness behavior. Brain Behav Immun Health 2021; 18: 100379.
14. Pollak Y, Ovadia H, Orion E, Weidenfeld J, Yirmiya R. The EAE-associated behavioral syndrome: I. Temporal correlation with inflammatory mediators. J Neuroimmunol 2003; 137 (1-2): 94-99.
15. Pollak Y, Ovadia H, Orion E, Yirmiya R. The EAE-associated behavioral syndrome: II. Modulation by anti-inflammatory treatments. J Neuroimmunol 2003; 137 (1-2): 100-108.
16. Kany S, Vollrath JT, Relja B. Cytokines in Inflammatory Disease. International Journal of Molecular Sciences 2019; 20 (23): 6008.
17. Lin CC, Edelson BT. New Insights into the Role of IL-1β in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis. J Immunol 2017; 198 (12): 4553-4560.
18. Bai Z, Chen D, Wang L, Zhao Y, Liu T, Yu Y et al. . Cerebrospinal Fluid and Blood Cytokines as Biomarkers for Multiple Sclerosis: A Systematic Review and Meta-Analysis of 226 Studies With 13,526 Multiple Sclerosis Patients. Front Neurosci 2019; 13: 1026.
19. Choy EHS, Calabrese LH. Neuroendocrine and neurophysiological effects of interleukin 6 in rheumatoid arthritis. Rheumatology (Oxford). 2018; 57 (11): 1885-1895.
20. Bårdsen K, Brede C, Kvivik I, Kvaløy JT, Jonsdottir K, Tjensvoll AB et al. Interleukin-1-related activity and hypocretin-1 in cerebrospinal fluid contribute to fatigue in primary Sjögren's syndrome. J Neuroinflammation. 2019; 16 (1): 102.
21. Feinstein A, Magalhaes S, Richard JF, Audet B, Moore C. The link between multiple sclerosis and depression. Nat Rev Neurol 2014; 10: 507-517.
22. Tarasiuk J, Kapica-Topczewska K, Czarnowska A, Chorąży M, Kochanowicz J, Kułakowska A. Co-occurrence of Fatigue and Depression in People With Multiple Sclerosis: A Mini-Review. Front Neurol 2022; 12: 817256.
23. Osimo EF, Pillinger T, Rodriguez IM, Khandaker GM, Pariante CM, Howes OD. Inflammatory markers in depression: A meta-analysis of mean differences and variability in 5,166 patients and 5,083 controls. Brain Behav Immun 2020; 87: 901-909.
24. Solaro C, Bergamaschi R, Rezzani C, Mueller M, Trabucco E, Bargiggia V, et al. Duloxetine is effective in treating depression in multiple sclerosis patients: an open-label multicenter study Clin Neuropharmacol 2013; 36: 114-116.
25. Govindarajan V, de Rivero Vaccari JP, Keane RW. Role of inflammasomes in multiple sclerosis and their potential as therapeutic targets. J Neuroinflammation 2020; 17: 260.
26. Ihim SA, Abubakar SD, Zian Z, Sasaki T, Saffarioun M, Maleknia S et al. Interleukin-18 cytokine in immunity, inflammation, and autoimmunity: Biological role in induction, regulation, and treatment. Front Immunol 2022; 13: 919973.
27. Arend WP, Palmer G, Gabay C. IL-1, IL-18, and IL-33 families of cytokines. Immunol Rev 2008; 223: 20-38.
28. Akita K, Ohtsuki T, Nukada Y, Tanimoto T, Namba M, Okura T et al. Involvement of caspase-1 and caspase-3 in the production and processing of mature human interleukin 18 in monocytic THP.1 cells. J Biol Chem 1997; 272: 26595-26603.
29. Fukaura H, Kikuchi S. [IL-18 in multiple sclerosis]. Nihon Rinsho. Japanese Journal of Clinical Medicine 2003; 61(8): 1416-1421.
30. Olcum M., Tastan B., Kiser C., Genc S., Genc K. Microglial NLRP3 inflammasome activation in multiple sclerosis. Advances in Protein Chemistry and Structural Biology 2020; 119: 247-308.
31. Miyoshi K, Obata K, Kondo T, Okamura H, Noguchi K: Interleukin-18-mediated microglia/astrocyte interaction in the spinal cord enhances neuropathic pain processing after nerve injury. J Neurosci 2008; 28: 12775-12787.
32. Kanai T, Kamada N, Hisamatsu T. Clinical strategies for the blockade of IL-18 in inflammatory bowel diseases. Curr Drug Targets 2013; 14: 1392-1399.
33. Shao XT, Feng L, Gu LJ, Wu LJ, Feng TT, Yang YM, et al. Expression of interleukin-18, IL-18BP, and IL-18R in serum, synovial fluid, and synovial tissue in patients with rheumatoid arthritis. Clin Exp Med 2009; 9: 215-221.
34. Shimizu M, Nakagishi Y, Yoshida A, Yachie A. Serum interleukin 18 as a diagnostic remission criterion in systemic juvenile idiopathic arthritis. J Rheumatol 2014; 41: 2328-2330.
35. Wu CY, Yang HY, Yao TC, Liu SH, Huang JL. Serum IL-18 as biomarker in predicting long-term renal outcome among pediatric-onset systemic lupus erythematosus patients. Medicine (Baltimore) 2016; 95: e5037.
36. Satış H, Özger HS, Aysert Yıldız P, Hızel K, Gulbahar Ö, Erbaş G et al. Prognostic value of interleukin-18 and its association with other inflammatory markers and disease severity in COVID-19. Cytokine 2021; 137: 155302.
37. Spitsin S, Koprowski H. Role of uric acid in multiple sclerosis. Curr Top Microbiol Immunol 2008; 318: 325-342.
38. Piancone F, Saresella M, Marventano I, La Rosa F, Santangelo MA, Caputo D et al. Monosodium Urate

Crystals Activate the Inflammasome in Primary Progressive Multiple Sclerosis. Front Immunol 2018; 9: 983.
39. Katarina V, Gordana T, Svetlana MD, Milica B. Oxidative stress and neuroinflammation should be both considered in the occurrence of fatigue and depression in multiple sclerosis. Acta Neurol Belg 2020; 120(4): 853-861.
40. Dantzer R, Kelley KW: Twenty years of research on cytokine-induced sickness behavior. Brain Behav Immun 2007; 21: 153-160.
41. Jahanbani-Ardakani H, Alsahebfosoul F, Etemadifar M, Abtahi SH. Interleukin 18 Polymorphisms and its serum level in Patients with Multiple Sclerosis. Ann Indian Acad Neurol 2019; 22 (4): 474-476.
42. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011; 69 (2): 292-302.
43. Lublin FD, Baier M, Cutter G. Effect of relapses on development of residual deficit in multiple sclerosis. Neurology 2003; 61 (11): 1528-1532.
44. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 1983; 33 (11): 1444-1452.
45. Minden SL, Feinstein A, Kalb RC, Miller D, Mohr DC, Patten SB et al. Guideline Development Subcommittee of the American Academy of Neurology. Evidence-based guideline: assessment and management of psychiatric disorders in individuals with MS: report of the Guideline Development Subcommittee of the American Academy of Neurology. Neurology 2014; 82 (2): 174-181.
46. Krupp LB, La Rocca NG, Muir-Nash J, Steinbery AD. The fatigue severity scales. Application to patients with multiple sclerosis and systemic lupus erythematosus. Arch Neurol 1989; 46 (10): 1121-1123.
47. Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. Annu Rev Immunol 2001; 19: 423-474.
48. Jander S, Stoll G. Differential induction of interleukin-12, interleukin-18, and interleukin-1beta converting enzyme mRNA in experimental autoimmune encephalomyelitis of the Lewis rat. J Neuroimmunol 1998; 91 (1-2): 93-99.
49. Gutcher I, Urich E, Wolter K, Prinz M, Becher B. Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation. Nat Immunol 2006; 7 (9): 946-953.
50. Karakas Celik S, Öz ZS, Dursun A, Unal A, Emre U, Cicek S et al. Interleukin 18 gene polymorphism is a risk factor for multiple sclerosis. Mol Biol Rep 2014; 41: 1653-1658.
51. Balashov KE, Rottman JB, Weiner HL, Hancock WW. CCR5(+) and CXCR3(+) T cells are increased in multiple sclerosis and their ligands MIP-1alpha and IP-10 are expressed in demyelinating brain lesions. Proc Natl Acad Sci 1999; 96(12): 6873-6878.
52. Wildbaum G, Youssef S, Grabie N, Karin N. Neutralizing antibodies to IFN-gamma-inducing factor prevent experimental autoimmune encephalomyelitis. J Immunol 1998; 161 (11): 6368-6374.
53. Chen YC, Chen SD, Miao L, Liu ZG, Li W, Zhao ZX et al. Serum levels of interleukin (IL)-18, IL-23 and IL-17 in Chinese patients with multiple sclerosis. J Neuroimmunol 2012; 243 (1-2): 56-60.
54. Nicoletti F, Di Marco R, Mangano K, Patti F, Reggio E, Nicoletti A et al. Increased serum levels of interleukin-18 in patients with multiple sclerosis. Neurology 2001; 57 (2): 342-344.
55. Huang WX, Huang P, Hillert J. Increased expression of caspase-1 and interleukin-18 in peripheral blood mononuclear cells in patients with multiple sclerosis. Multiple Sclerosis Journal 2004; 10 (5): 482-487.
56. Losy J. and Niezgoda A. IL-18 in patients with multiple sclerosis. Acta Neurologica Scandinavica 2001; 104: 171-173.
57. Trenova AG, Slavov GS, Draganova-Filipova MN, Mateva NG, Manova MG, Miteva LD et al. Circulating levels of interleukin-17A, tumor necrosis factor-alpha, interleukin-18, interleukin-10, and cognitive performance of patients with relapsing-remitting multiple sclerosis. Neurol Res 2018; 40 (3): 153-159.
58. Yang JW, Mao B, Tao RJ, Fan LC, Lu HW, Ge BX, Xu JF. Corticosteroids alleviate lipopolysaccharide-induced inflammation and lung injury via inhibiting NLRP3-inflammasome activation. J Cell Mol Med. 2020; 24(21): 12716-12725.
59. Wu L, Zhou C, Wu J, Chen S, Tian Z, Du Q. Corticosterone Inhibits LPS-Induced NLRP3 Inflammasome Priming in Macrophages by Suppressing Xanthine Oxidase. Mediators Inflamm. 2020; 2020: 6959741.
60. Soares JL, Oliveira EM, Pontillo A. Variants in NLRP3 and NLRC4 inflammasome associate with susceptibility and severity of multiple sclerosis. Mult Scler Relat Disord. 2019; 29: 26-34.
61. Yamanishi K, Doe N, Mukai K, Ikubo K, Hashimoto T, Uwa N et al. Interleukin-18-deficient mice develop hippocampal abnormalities related to possible depressive-like behaviors. Neuroscience 2019; 408: 147-160.
62. Yamanishi K, Doe N, Mukai K, Hashimoto T, Gamachi N, Hata M et al. Acute stress induces severe neural inflammation and overactivation of glucocorticoid signaling in interleukin-18-deficient mice. Transl Psychiatry 2022; 12 (1): 404.
63. Wu D, Zhang G, Zhao C, Yang Y, Miao Z, Xu X. Interleukin-18 from neurons and microglia mediates depressive behaviors in mice with post-stroke depression. Brain Behav Immun 2020; 88: 411-420.
64. Haastrup E, Bukh JD, Bock C, Vinberg M, Thørner LW, Hansen T et al. Promoter variants in IL18 are associated with onset of depression in patients previously exposed to stressful-life events. J Affect Disord 2012; 136 (1-2): 134-138.
65. Merendino RA, Di Rosa AE, Di Pasquale G, Minciullo PL, Mangraviti C, Costantino A et al. Interleukin-18 and CD30 serum levels in patients with moderate-severe depression. Mediators Inflamm 2002; 11 (4): 265-267.

66. Alcocer-Gomez E, de Miguel M, Casas-Barquero N, Nunez-Vasco J, Sanchez-Alcazar JA, Fernandez-Rodriguez A et al. NLRP3 inflammasome is activated in mononuclear blood cells from patients with major depressive disorder. Brain Behav Immun 2014; 36: 111-117.
67. Al-Hakeim HK, Al-Rammahi DA, Al-Dujaili AH. IL-6, IL-18, sIL-2R, and TNFalpha proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation. J Affect Disord 2015; 182: 106-114.
68. Du X, Zou S, Yue Y, Fang X, Wu Y, Wu S et al. Peripheral Interleukin-18 is negatively correlated with abnormal brain activity in patients with depression: a resting-state fMRI study. BMC Psychiatry 2022; 22 (1): 531.
69. Zhao N, Dong C. Correlation of Serum IL-18, BDNF, and IL-1β with Depression and Prognosis after Acute Exacerbation of Chronic Obstructive Pulmonary Disease. Comput Math Methods Med. 2022; 2022:3555982. Retraction in: Comput Math Methods Med 2022; 2022:9893716.
70. Wedervang-Resell K, Friis S, Lonning V, Smelror RE, Johannessen C, Reponen EJ et al. Increased interleukin 18 activity in adolescents with early-onset psychosis is associated with cortisol and depressive symptoms. Psychoneuroendocrinology 2020; 112: 104513.
71. Liu CH, Zhang GZ, Li B, Li M, Woelfer M, Walter M, Wang L. Role of inflammation in depression relapse. J Neuroinflammation 2019; 16(1): 90.
72. Herman FJ, Pasinetti GM. Principles of inflammasome priming and inhibition: Implications for psychiatric disorders. Brain Behav Immun 2018; 73:66-84
73. 73. Al-Hakeim HK, Twayej AJ, Al- Dujaili AH. Reduction in serum IL-1β, IL-6, and IL-18 levels and Beck Depression Inventory-II score by combined sertraline and ketoprofen administration in major depressive disorder: A clinical trial. Neurol Psychiatry Brain Res 2018; 30: 148-153.

CLINICAL FOOT FINDINGS AND PLANTAR PRESSURE ASSESSMENT IN TYPE 2 DIABETES: A CROSS-SECTIONAL COMPARISON OF STATIC AND DYNAMIC MEASURES

Dragana Bubanja[1,2], Filip Mihajlovic[3] and Irfan Corovic[4,5]

[1]*University Clinical Center Kragujevac, Clinic for Internal Medicine, Kragujevac, Serbia*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Internal Medicine, Kragujevac, Serbia*
[3]*University of Kragujevac, Faculty of Medical Sciences, Department of Forensic Medicine, Kragujevac, Serbia*
[4]*General Hospital Novi Pazar, Novi Pazar, Serbia*
[5]*Center for Molecular Medicine and Stem Cell Research, University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia*

Received: 11.02.2026.
Accepted: 03.04.2026.

Corresponding author:

Filip Mihajlovic, PhD candidate

University of Kragujevac, Faculty of Medical Sciences, Department of Forensic Medicine, Kragujevac, Serbia

E-mail: f.mihajlovic@yahoo.com

UDK:

Eabr 2026; 27(2):159-166
DOI:10.2478/eabr-2026-0012

ABSTRACT

Among chronic complications of diabetes mellitus, diabetic foot remains one of the most challenging conditions in clinical practice. Increased and repetitive plantar pressure represents an important etiological factor which, together with neurological, dermatological, musculoskeletal, and vascular components, contributes to the development of plantar tissue damage in people with diabetes. The aim of this study was to assess the diagnostic agreement between commonly used clinical and instrumental methods for the detection of minor and major plantar foot lesions, including the monofilament test and static and dynamic plantar pressure measurement models. This cross-sectional observational study examined both feet of each participant. Minor and major plantar lesions were recorded during clinical foot examination, a 16-point monofilament test was performed, and static and dynamic plantar pressure measurements were obtained for both feet. Each foot underwent three static and three dynamic measurements, resulting in a total of 1,560 measurements. Dynamic plantar pressure measurements showed a higher level of agreement with clinical foot examination compared with static plantar pressure measurements and monofilament testing. Dynamic plantar pressure assessment may represent a useful adjunct method for identifying areas of increased mechanical load on the plantar surface of the foot. However, further longitudinal studies are required to determine its potential clinical value in diabetic foot care.

Keywords: *Diabetic foot, diabetes mellitus, plantar pressure, monofilament test.*

INTRODUCTION

Diabetic foot disease involves one or more foot disorders in people with diabetes mellitus, including peripheral polyneuropathy, peripheral vascular disease, infection, ulceration, neuroosteoarthropathy, gangrene, or amputation. Although this complication affects a substantial proportion of people with diabetes, it represents not only a personal burden for the affected individual but also a significant burden for the family and healthcare system [1]. Once a foot ulcer develops, the risk of recurrent ulceration and lower-limb amputation increases considerably, creating a major medical, social, and economic problem. Therefore, early identification of factors associated with plantar tissue damage is of particular clinical importance [2, 3].

Plantar pressure is defined as the force applied per unit area by the plantar surface of the foot to the ground. Increased and repetitive plantar pressure contributes to the development of microlesions on the plantar surface, which may be detected during routine clinical examination. Plantar pressure is commonly assessed using baropodographic platforms, which provide quantitative information on pressure distribution under static and dynamic conditions [4]. Clinical examination of the foot represents the primary reference method in routine practice, while plantar pressure measurements and sensory testing are considered useful adjunct tools for identifying areas exposed to increased mechanical load [5]. Plantar pressure may increase during both standing and walking. Depending on the depth and extent of tissue damage, clinical manifestations may involve the epidermis, dermis, subcutaneous tissue, tendons, joint ligaments, or plantar fascia, and in advanced stages, joints and bones may also be affected [6]. Most countries apply international guidelines for diabetic foot management and operate specialized multidisciplinary clinics. Nevertheless, there remains a need for practical and objective methods that can support routine clinical assessment and contribute to the early detection of plantar tissue changes [7, 8].

The aim of this study was to assess the diagnostic agreement between commonly used clinical and instrumental methods for the detection of minor and major plantar foot lesions, including the monofilament test, static plantar pressure measurement, and dynamic plantar pressure measurement.

MATERIALS AND METHODS

Study design

This was a clinical, non-interventional, cross-sectional observational study including participants from an study group and a control group. The study was approved by the Ethics Committee of the Clinical Center Kragujevac and conducted at the Center for Endocrinology, Diabetes, and Metabolic Diseases of the University Clinical Center Kragujevac.

Study population

Inclusion criteria

Participants were recruited using a convenience sampling approach and included individuals who provided written informed consent, were older than 18 years, and had a body mass index (BMI) below 35 kg/m². The study group consisted of patients with type 2 diabetes mellitus and sensorimotor diabetic polyneuropathy, diagnosed according to standard criteria as the presence of at least one neuropathic symptom or clinical sign in combination with abnormal nerve conduction study findings, after exclusion of other identifiable causes of peripheral neuropathy. The control group consisted of healthy volunteers without diabetes mellitus.

Exclusion criteria

The following participants were excluded from the study: patients with other neurological disorders relevant to the research, including cerebrovascular stroke, transient ischemic attack, myelodegenerative diseases, demyelinating disorders of the nervous system, and tumors of the nervous system; patients with orthopedic disorders, fractures or deformities of the spine or foot; patients with rheumatoid arthritis and foot deformities; patients with limb amputation; patients with thyroid dysfunction, liver or kidney insufficiency; as well as patients receiving opioid analgesic therapy, individuals with opioid dependence, and patients with mental disorders affecting perception.

Study procedures

All measurements and examinations were performed during a single visit for each participant. Clinical foot examination included a general assessment of foot condition, careful visual inspection immediately after removal of footwear and socks, in order to identify areas of increased pressure related to inadequate footwear and existing deformities of the toes or foot. Minor and major diabetic foot lesions corresponding to high-pressure areas were recorded, including rhagades, calluses, hyperkeratosis, and non-infected plantar ulcers. Each foot was photographed and independently examined by two endocrinologists. Both examiners documented the location and type of lesion on a standardized schematic foot chart.

Sensory testing was performed using a 10 g Semmes-Weinstein monofilament at eight standardized points on each foot: the first, third, and fifth metatarsal heads, the plantar surface of the hallux, third and fifth toes, the heel area, and the dorsal surface of the hallux. The monofilament was applied perpendicular to the skin and pressure was gradually increased until the filament bent into a C shape, which was maintained for one second. Participants were asked to indicate whether they perceived the stimulus. The monofilament score ranged from 0 to 16 and represented the number of plantar sites at which the stimulus was correctly perceived.

Static and dynamic plantar pressure measurements were performed using a baropodographic platform (FOOTWORK PRO, France), equipped with 4096 calibrated sensors (two sensors per cm²). The active surface of the platform measured 490 × 490 mm and allowed assessment of pressure distribution and force during standing and walking. All measurements were performed in real time. For each participant, three static and three dynamic measurements were performed separately for each foot. The static model measured plantar pressure during standing. Participants stood barefoot on the platform in an upright position, with feet shoulder-width apart and gaze directed forward, for 30 seconds. Three consecutive measurements were obtained, with a five-minute rest period between measurements, during which participants stepped off the platform and resumed the initial position. The dynamic model recorded plantar pressure during walking using the three-step protocol. Participants started three steps away from the platform (0.75-1.5 m), initiated walking with the left foot, and stepped onto the platform with the left foot on the third step. The procedure was then repeated starting with the right foot. This sequence was repeated three times, resulting in three dynamic measurements for each foot. Variables recorded included maximum and mean plantar pressure under static and dynamic conditions, contact time between foot and platform, and pressure-time integral for each foot. Pressure-time integral values were expressed in units provided by the device software (kPa·ms). Clinical findings at the eight predefined plantar points were compared with monofilament test results and corresponding parameters from static and dynamic plantar pressure measurements. Percentage agreement between methods was calculated for both feet. For the purpose of this study, calluses and fissures were defined broadly as any visible hyperkeratosis or superficial skin discontinuity, regardless of size or clinical significance.

Statistical analysis

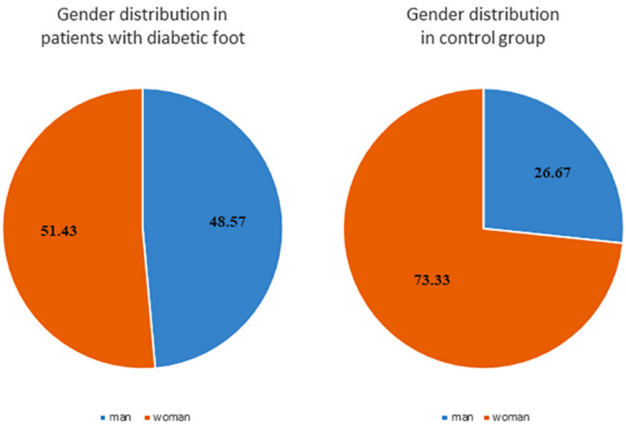
Statistical analysis was performed using IBM SPSS Statistics version 20. Data were presented graphically and in tables. Descriptive statistics included frequencies, measures of central tendency (mode, median, and mean), and measures of variability (quantiles, variance, and standard deviation). Group comparisons were performed using the independent samples t-test or the Mann-Whitney U test, depending on data distribution. Paired comparisons within the same participant under different conditions were performed using the paired samples t-test or the Wilcoxon signed-rank test, as appropriate.

RESULTS

Demographic and anthropometric characteristics

A total of 130 participants of the same ethnic background were included in the study. The study group consisted of 70 participants, including 36 women (51.43%) and 34 men (48.57%). The control group included 60 participants, of whom 44 were women (73.33%) and 16 were men (26.67%). The mean age of participants in both groups was 54.56 ± 14.22 years. The distribution of participants by sex is presented in Figure 1.

Figure 1. Distribution of subjects by gender



Agreement between clinical findings and diagnostic methods

By comparing clinical findings on the plantar surface of the foot with positive results obtained from static and dynamic plantar pressure measurements and monofilament testing, the percentage agreement among the three methods was determined. During static measurement, the position of each foot was automatically displayed in centimeters, and the software calculated pressure distribution for both feet, including forefoot and rearfoot regions, as well as maximum and mean plantar pressure values. The same plantar points were compared for monofilament testing and for maximum pressure values obtained from static and dynamic measurements.

Monofilament testing

Monofilament testing was considered positive when the participant failed to perceive the stimulus at one of the sixteen tested points (eight points on each foot). The results showed that the monofilament test had a specificity of 100% and a positive predictive value of 100%, while sensitivity was 61.43% and negative predictive value was 69% in the detection of diabetic foot lesions. The derived cut-off value in the study group was below 11 (Table 1).

Clinical findings

Microlesions on the plantar surface of the foot were identified in the form of calluses, rhagades, and fissures. Rhagade-type lesions were observed in 36 participants (51.42%) on both feet. Calluses were present in 45 participants (64.3%) on the right foot and in 39 participants (55.7%) on the left foot (Table 2). Minor plantar skin changes were frequent in both groups due to the broad operational definition applied, which included minimal hyperkeratosis/superficial fissuring.

Macrolesions in the form of non-infected plantar ulcers were detected in 23 participants (32.85%) at the time of examination. A history of previous plantar ulcers was reported

in 47 participants (67.15%) from the study group. No plantar ulcers were observed in the control group. A statistically significant difference between the study and control groups was found for the presence of plantar ulcers ($p < 0.001$; Table 3).

Static plantar pressure measurements

In the static plantar pressure measurement model, the following parameters were analyzed: mean pressure of both feet, mean pressure of the dominant foot, and combined mean pressure of both feet (Table 4). No statistically significant differences were observed between the study and control groups, except for mean pressure of the right foot ($p = 0.021$). Maximum pressure on the dominant foot showed higher values in the study group, but without statistical significance.

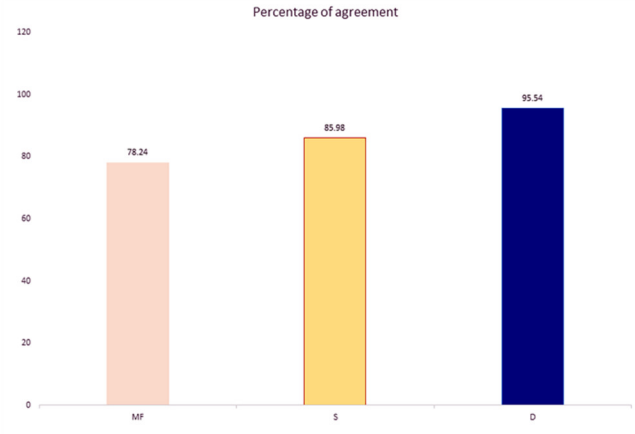
Dynamic plantar pressure measurements

In the dynamic measurement model, mean plantar pressure and maximum plantar pressure at the same points on each foot were analyzed. No statistically significant differences were found between the study and control groups for these parameters. However, the pressure-time integral parameter was significantly higher in participants with diabetic foot compared with the control group, including mean, dominant, and total values for both feet (Table 5).

Percentage agreement between methods

Clinical examination identified micro- and macrolesions on the plantar surface of the foot, which were compared with positive findings from static and dynamic plantar pressure measurements and monofilament testing. The percentage agreement among the three methods was calculated. The highest agreement was observed for dynamic plantar pressure measurements across sixteen plantar points, with the smallest standard error and the narrowest range. The lowest agreement was observed for monofilament testing (Table 6, Figure 2).

Figure 2. Comparison results of monofilament tests, static and dynamic models measurement of plantar pressures with clinical findings



MF- monofilament test, S- static measurement model, D- dynamic plantar pressure

The dynamic plantar pressure measurement model showed the highest percentage agreement with clinical examination (95.54%), followed by the static measurement model (85.08%) and the monofilament test (78.24%).

DISCUSSION

Foot ulcer occurrence in people with diabetes mellitus remains frequent despite the numerous diagnostic methods and preventive measures introduced over recent decades. This complication may lead to lower limb amputation and represents not only a medical, psychological, and physical burden for the affected individual, but also a social and economic burden for both the patient and society [9]. The earliest clinical manifestations of increased plantar pressure appear as microlesions that may progress toward ulcer formation. In our study, examination of plantar microlesions revealed changes such as calluses, rhagades, and fissures. Rhagade-type lesions were observed in 36 participants (51.42%) on both feet, while calluses were present in 45 participants (64.3%) on the right foot and in 39 participants (55.7%) on the left foot. Macrolesions in the form of non-infected plantar ulcers were detected in 23 participants (32.85%) at the time of examination (Table 2).

According to published data, approximately 50% to 60% of diabetic foot ulcers become infected, and about 20% of moderate to severe infections lead to lower limb amputation [9]. The five-year mortality rate among individuals with diabetic foot ulcers is approximately 30%, and exceeds 70% in those who undergo major amputation. Mortality among people with diabetic foot ulcers reaches 231 deaths per 1000 individuals per year, compared with 182 deaths per 1000 individuals with diabetes without foot ulceration [9]. Diabetic peripheral neuropathy is considered one of the main risk factors for the development of foot ulcers. According to the 2023 guidelines of the International Working Group on the Diabetic Foot (IWGDF), neuropathy should be systematically assessed using standardized tools such as vibration perception threshold testing, monofilament testing, and symptom questionnaires [10].

Prevention of foot ulcer development in people with diabetes and diabetic peripheral neuropathy remains of major clinical importance, as the first ulcer markedly increases the risk of new ulcer formation on the same or contralateral limb [11]. This process is related to altered foot architecture caused by neuropathy. Various toe and foot deformities tend to worsen after partial amputations, promoting new ulcer formation on the same foot after minor amputation or on the opposite limb after major amputation [12]. Elevated plantar pressure represents another important factor in the etiology of diabetic foot. Over recent decades, diabetic foot has received increasing scientific attention, and multiple methods have been developed to assess plantar pressure [13]. However, most previous studies and meta-analyses have focused primarily on treatment strategies rather than on objective assessment methods supporting early identification of high-risk plantar regions [14-16].

Table 1. Diagnostic performance of the monofilament score for detecting plantar foot lesion

AUC	SE	95% CI	z	p
0.82	0.0319	0.743 - 0.882	10.027	< 0.001
"Cut point"	SN (%)	SP (%)	PPV (%)	NPV (%)
< 11	61.43	100	100	69

AUC - area under the curve "Cut point" - criterion value, SN - sensitivity, SP -specificity, PPV - positive predictive value, NPV - negative predictive value, p - statistical significance

Table 2. Display of the frequency of microlesions on the plantar side of the foot

Microlesions foot	Right leg		Left leg	
Study population	Contro group (n = 60)	Study group (n = 70)	Control group (n = 60)	Study group (n = 70)
Cracks				
yes	0 (0%)	34 (48.58%)	0 (0%)	34 (48.58 %)
no	60 (100%)	36 (51.42%)	60 (100%)	36 (51.42%)
Calluses				
yes	0 (0%)	25 (35.7%)	9 (15%)	31 (44.28%)
no	60 (100%)	45 (64.3%)	51 (85%)	39 (55.71%)

Table 3. Ulcers on the plantar side of the foot

History of existence ulcer	Control group (n = 60)	Study group (n = 70)	Total
negative	60 (100%)	47 (67.15%)	107
positive	0 (0%)	23 (32.85%)	23

Table 4. Plantar average pressures during static measurement

Average pressure (kPa)	Control group (n = 60) Md	Study group (n = 70) Md	U	z	p
P 1	18.810	19.078	2008.5	0.260	p > 0.05
Pd	20.180	19.005	1578.5	2.300	p = 0.021
P_{max}	63.720	75.870	1761.0	1.437	p > 0.05
(Pl+Pd)/2	20.010	18.975	1942.5	0.579	p > 0.05
P 1 /Pd	21.270	21.460	2042.0	0.109	p > 0.05
P 1 and Pd	19.715	19.010	7509.5	1.257	p > 0.05

P_l - average pressure of the left foot during static measurement, Pd - average pressure of the right foot during static measurement, Pmax maximum pressure during static measurement on the left or right foot, Md - median, p - statistical significance.

Table 5. Integral pressure/time for both feet during dynamic measurement

Integral pressure/time (kPa·ms)	Control group (number of measurements)	Study group (number of measurements)	Control group (n = 60) Md	Studygroup (n = 70) Md	U	z	p
I l	60	70	971.820	1088.47	1674	-1.849	p = 0.06
Id	60	70	930.320	1097.67	1676	-1.839	p = 0.07

Integral pressure/time (kPa·ms)	Control group (number of measurements)	Study group (number of measurements)	Control group (n = 60) Md	Studygroup (n = 70) Md	U	z	p
(Il+Id)/2	60	70	944.770	1055.83	1652	-1.953	p = 0.05
Il or Id	60	70	1066.49	1293.72	1618	-2.113	p = 0.03
Il + Id	120	140	947.695	1088.47	6698	-2.616	p < 0.01

I_l - integral pressure/time for the left foot in the dynamic measurement model**, Id -** integral pressure/time for the right foot in the dynamic measurement model, **(Il+Id)/2** - mean values integral pressure time**, Il or Id** - dominant integral pressure time in the dynamic model pressure measurement, **Il + Id** - total integral pressure time

Table 6. Comparison of monofilament test, static and dynamic measurement model of plantar pressures

	Monofilament	Static measurement	Dynamic measurement
Number of subjects	130	130	130
Mean value (%)	78.2402	85.9787	95.5426
Median (%)	81.2500	87.5000	93.7500
Standard error (%)	18.47607	12.20248	5.36701
Range (%)	75.00	96.25	25.00
Minimum (%)	25.00	3.75	75.00
Maximum (%)	100.00	100.00	100.00

To date, limited data are available from studies that directly compare commonly used diagnostic tests and plantar pressure measurements with clinical foot findings in a cross-sectional manner. The age of participants in our study was comparable to age groups reported in other investigations, with a mean age of 54.56 ± 14.22 years. The mean duration of diabetes in the study group was 13.86 ± 7.85 years. Previous studies have shown that individuals with diabetes lasting longer than ten years have a substantially increased risk of lower limb amputation. Distal symmetric polyneuropathy represents the most common form of diabetic neuropathy and typically shows slow progression [17].

In the present study, dynamic plantar pressure measurements showed a higher percentage agreement with clinical foot examination compared with static measurements and monofilament testing (Figure 1). Other studies have attempted to improve static plantar pressure assessment using machine learning approaches. For example, Sheikh et al. [18] reported improved accuracy under static conditions using gradient boosting techniques, while Gerlein et al. [19] reported lower accuracy using similar methods. Regional segmentation of the forefoot and rearfoot has been proposed as a way to enhance the discriminatory capacity of static measurements. Armstrong et al. [20] demonstrated that region-specific pressure analysis may improve identification of ulcer-prone areas; however, their study did not include neuropathy classification or direct clinical examination findings, unlike the present study.

Some investigations, including studies conducted in Japanese populations, reported significantly higher plantar pressure values and pressure-time integrals in patients with diabetic peripheral neuropathy compared with patients with diabetes without neuropathy [21]. These findings support the potential relevance of dynamic plantar pressure parameters, particularly pressure-time integral, in identifying plantar regions exposed to prolonged mechanical stress during gait. However, other studies reported inconsistent results, especially when using baropodographic platforms, and suggested that in-shoe measurement systems may provide greater precision. Recent technological developments include systems for dynamic pressure redistribution, such as pneumatically driven mechanisms and magnetorheological fluid-based systems, which aim to regulate plantar pressure within recommended thresholds. When integrated into footwear or insoles, these technologies may contribute to reducing mechanical stress and supporting diabetic foot care [6, 22].

The main limitations of most existing studies include relatively small sample sizes and cross-sectional designs. Although diabetic neuropathy diagnosis was confirmed using combined clinical and electrophysiological criteria, detailed stratification according to neuropathy severity, diabetes duration, and glycemic control was not performed. The absence of subgroup analyses based on neuropathy severity scales or metabolic control parameters may have limited the ability to detect potential gradations in plantar pressure patterns. Future studies incorporating standardized neuropathy severity scoring systems and metabolic profiling are warranted to better define the relationship between mechanical load

distribution and neuropathy progression. Additionally, novel technological solutions remain limited in their implementation, particularly in healthcare systems where primary prevention of diabetic foot has not yet been fully established [23, 24]. The cross-sectional nature of the present study does not allow conclusions regarding causality or prediction of future ulcer development, but provides descriptive information on the agreement between clinical findings and commonly used assessment methods.

CONCLUSION

The results of this study showed that dynamic plantar pressure measurements demonstrated a higher level of agreement with clinical foot examination compared with static measurements and monofilament testing. Plantar pressure mapping may represent a useful tool for identifying areas of increased mechanical load on the plantar surface of the foot.

This approach may support clinical assessment by contributing to individualized orthotic planning, objective monitoring of plantar pressure distribution, and evaluation of therapeutic interventions. However, due to the cross-sectional design of the study, no conclusions can be drawn regarding causality or prediction of future ulcer development.

Further longitudinal studies with standardized protocols are required to confirm the clinical relevance of dynamic plantar pressure assessment in diabetic foot care.

REFERENCES

1. Lockhart M, Dinneen SF, O'Keeffe DT. Plantar pressure measurement in diabetic foot disease: A scoping review. J Diabetes Investig. 2024. 15(8):990-999.
2. Kosaji D, Awad MI, Katmah R, et al. Diabetic foot prevention, assessment, and management using innovative smart wearable technology: a systematic review. J Neuroeng Rehabil. 2025; 22(1):168.
3. Matijevich E, Minty E, Bray E, Bachus C, Hajizadeh M, Liden B. A Multi-Faceted Digital Health Solution for Monitoring and Managing Diabetic Foot Ulcer Risk: A Case Series. Sensors (Basel). 2024; 24(9):2675.
4. Castro-Martins P, Marques A, Coelho L, Vaz M, Costa JT. Plantar pressure thresholds as a strategy to prevent diabetic foot ulcers: A systematic review. Heliyon. 2024;10(4):e26161.
5. Castro-Martins P, Pinto-Coelho L, Campilho RDSG. Calibration and Modeling of the Semmes-Weinstein Monofilament for Diabetic Foot Management. Bioengineering (Basel). 2024;11(9):886.
6. Castro-Martins P, Marques A, Pinto-Coelho L, Fonseca P, Vaz M. A Portable Insole System for Actively Controlled Offloading of Plantar Pressure for Diabetic Foot Care. Sensors (Basel). 2025; 25(12):3820.
7. Erel V, Nasirian A, Gu Y, Lavery L, Wijesundara MBJ. Development of Cyclic Pressure Offloading Insole for Diabetic Foot Ulcer Prevention. Int J Low Extrem Wounds. 2024:15347346241234825.
8. Bus SA. Preventing foot ulcers in diabetes using plantar pressure feedback. Lancet Digit Health. 2019 Oct;1(6):e250-e251.
9. Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic Foot Ulcers: A Review. JAMA. 2023; 330(1):62-75.
10. Monteiro-Soares M, Hamilton EJ, Russell DA, et al. Guidelines on the classification of foot ulcers in people with diabetes (IWGDF 2023 update). Diabetes Metab Res Rev. 2024; 40(3):e3648.
11. Zhang Y, Huang B, Wang T, Dong H, Huang X, Li X. A systematic review and meta-analysis of treatment modalities and their impact on the healing progression of diabetic foot ulcers. Int J Burns Trauma. 2025; 15(2):41-52.
12. Wong DW, Chow EM, Liyeung LL, Wang J, Mak TC, Cheung JC, Ni M, Leung AK. Does Hallux Valgus Impair Medial Forefoot Loading? A Meta-Analysis of Plantar Pressure Distribution. J Foot Ankle Res. 2025; 18(3):e70073.
13. Wang Q, Guan H, Wang C, et al. A wireless, self-powered smart insole for gait monitoring and recognition via nonlinear synergistic pressure sensing. Sci Adv. 2025;11(16):eadu1598.
14. Hu X, Meng H, Liang J, et al. Comparison of the efficacy of 12 interventions in the treatment of diabetic foot ulcers: a network meta-analysis. PeerJ. 2025; 13:e19809.
15. Coye TL, Bargas Ochoa M, Zulbaran-Rojas A, et al. Healing of diabetic neuropathic foot ulcers receiving standard treatment in randomised controlled trials: A random effects meta-analysis. Wound Repair Regen. 2025; 33(1):e13237.
16. Angulo AR, Caballero-Alvarado J, Sarmiento-Falen J, Zavaleta-Corvera C. Comparative Assessment of Negative Pressure Wound Therapy Versus Standard Treatment in Diabetic Foot Ulcers: A Systematic Review and Meta-Analysis. J Wound Ostomy Continence Nurs. 2025; 52(3):227-238.
17. Pasnoor M, Dimachkie MM, Kluding P, Barohn RJ. Diabetic neuropathy part 1: overview and symmetric phenotypes. Neurol Clin. 2013. 31(2):425-45.
18. Sheikh MM, Balachandra M, V G N, Maiya AG. Predicting diabetic peripheral neuropathy through advanced plantar pressure analysis: a machine learning approach. Sci Rep. 2025 Jul 1;15(1):20962. doi: 10.1038/s41598-025-07774-0. PMID: 40595132; PMCID: PMC12215299.
19. Gerlein EA, Calderón F, Zequera-Díaz M, Naemi R. Can the Plantar Pressure and Temperature Data Trend Show the Presence of Diabetes? A Comparative Study of a Variety of Machine Learning Techniques. Algorithms. 2024; 17(11):519.
20. Armstrong DG, Holtz-Neiderer K, Wendel C, Mohler MJ, Kimbriel HR, Lavery LA. Skin temperature monitoring reduces the risk for diabetic foot ulceration in high-risk patients. Am J Med. 2007; 120(12):1042-6.
21. Mima S, Abe Y, Yamasaki H, Bando M, et al. Plantar pressure and shear stress during gait in people with diabetic neuropathy. Diabetol Int. 2025; 16(2):285-293.

22. Hemler SL, Ntella SL, Jeanmonod K, et l. Intelligent plantar pressure offloading for the prevention of diabetic foot ulcers and amputations. Front Endocrinol (Lausanne). 2023; 14:1166513.
23. Zhang J, Lin J, Wu L, Lin J. Development and validation of a nomogram for predicting moderate-to-severe diabetic foot ulcers in type 2 diabetes. Front Endocrinol (Lausanne). 2025; 16:1669605.
24. Ramadhan GT, Haris F, Jan YK, et al. Effect of different inner pressures of air insoles and walking durations on plantar pressure time integral. Sci Rep. 2024; 14(1):19272.

CHROMATOGRAPHIC RETENTION OF NAPROXEN-BASED THIOUREA DERIVATIVES IN A MICELLAR SYSTEM AS A PREDICTOR OF PASSIVE GASTROINTESTINAL ABSORPTION

Nikola Nedeljkovic[1,*], Vladimir Dobricic[2,*], Marina Vesovic[1], Stefan Gavrilovic[1], Zorica Vujic[2] and Milos Nikolic[1]

[1] *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Kragujevac, Serbia*
[2] *University of Belgrade - Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Belgrade, Serbia*
* These authors contributed equally to this work

Received: 11.02.2026.
Accepted: 13.04.2026.

Corresponding author:

Nikola Nedeljkovic

University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy, Svetozara Markovića 69, 34000 Kragujevac, Serbia

E-mail: nikola.nedeljkovic@fmn.kg.ac.rs

UDK:

Eabr 2026; 27(2):167-172
DOI:10.2478/eabr-2026-0017

ABSTRACT

Experimental assessment of passive gastrointestinal absorption is essential in early drug profiling. Biopartitioning micellar chromatography (BMC), a modified micellar liquid chromatography technique employing nonionic surfactants above the critical micellar concentration, enables indirect assessment of passive permeability by reflecting solute distribution among the stationary, aqueous, and micellar phases. BMC was employed to investigate the retention behavior and biorelevant properties of seven newly synthesized naproxen-based thiourea derivatives under biomimetic conditions mimicking biological membranes. Chromatographic experiments were performed using Brij 35 as a nonionic surfactant. To further elucidate structure-retention relationships, quantitative structure-retention relationship (QSRR) analysis was conducted using multiple linear regression. The results demonstrated pronounced differences in retention behavior among the tested compounds, primarily governed by their structural features. Derivatives containing esterified aromatic amino acids exhibited the highest retention factors, suggesting enhanced passive gastrointestinal absorption, whereas the p-aminoacetophenone derivative showed the lowest retention and absorption potential. A statistically significant model was obtained with the Mor20u descriptor, achieving high correlation (R = 0.977) between the experimental retention factor (k) values and those predicted by the MLR model. The selected 3D-MoRSE descriptor effectively captured the influence of molecular polarizability and aromaticity on retention behavior. However, these findings should be interpreted with caution given that the analysis was based on a limited dataset comprising only seven compounds. Overall, the combined BMC and QSRR approach provides valuable insights into the biopharmaceutical properties of naproxen-based thiourea derivatives and identified structural features associated with favorable passive gastrointestinal absorption.

Keywords: *Naproxen, thiourea derivatives, BMC, QSRR.*

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed for treating pain, inflammation, and fever, with therapeutic action arising from cyclooxygenase (COX) inhibition and reduced prostaglandin synthesis (1). Among them, naproxen is a propionic acid derivative with well-documented clinical efficacy, particularly in musculoskeletal disorders and dysmenorrhea (2). Despite this, structural modification of naproxen remains a relevant medicinal chemistry strategy for improving biological profiles, minimizing adverse effects, and modulating physicochemical and biopharmaceutical characteristics. Thiourea derivatives constitute a versatile chemical class displaying anti-inflammatory, anticancer, antimicrobial, and enzyme inhibitory effects due to their ability to establish hydrogen bonding and conformational interactions with biological targets (3,4).

Naproxen-based thiourea compounds have been synthesized with the aim of retaining key pharmacophoric elements of the parent drug while introducing new substituents that potentially alter selectivity or biological activity. The results indicate that these derivatives may modulate COX-2 or 5-lipoxygenase (5-LOX) activity, as well as exhibit cytotoxic effects against selected tumor cell lines (5,6). These structural modifications generate compounds with altered lipophilicity or hydrogen bonding patterns.

Considering that NSAIDs are predominantly administered orally, understanding the gastrointestinal absorption of these derivatives is essential to anticipate their in vivo performance. For orally administered drugs, passive transcellular diffusion represents the predominant intestinal absorption mechanism for compounds without carrier-mediated transport (7,8). Experimental assessment of passive permeability therefore plays a central role in early drug profiling. Several in vitro and in silico tools have been established to estimate passive gastrointestinal absorption. However, chromatographic biomimetic systems have gained attention due to their reproducibility, low material consumption, and capability to mimic membrane-like environments without reliance on biological systems. Biopartitioning micellar chromatography (BMC) is a modified micellar chromatographic technique in which nonionic surfactants are added to the aqueous mobile phase at concentrations exceeding the critical micellar concentration (9,10). In BMC systems, surfactant monomers adsorb onto the hydrophobic stationary phase, while excess monomers form micelles within the mobile phase. This arrangement mimics structural features of biological membranes: hydrophobic surfactant chains resemble phospholipid tails, polar headgroups simulate membrane interfaces, and micellar aggregates approximate extracellular aqueous domains (9-11). Under these conditions, solute retention reflects distribution between stationary phase, aqueous phase, and micellar phase, providing an indirect measure of passive permeability and membrane partitioning (Figure 1). The usefulness of the BMC method in predicting drug permeation across biological barriers, including the blood-brain barrier, skin, and the gastrointestinal tract, has been demonstrated (10-12).

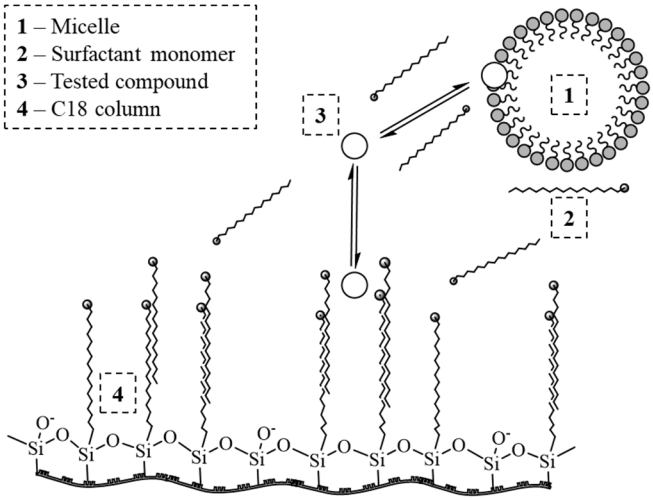


Figure 1.Schematic illustration of the micellar chromatography mechanism

Beyond chromatographic assessment, chemometric modeling provides further insight into structural determinants of retention and permeability. Quantitative structure-retention relationship (QSRR) analysis correlates chromatographic retention with molecular descriptors that encode physicochemical, geometric, or electronic features (13,14). QSRR models allow the identification of structural features influencing retention in biomimetic systems and facilitate computational prediction of chromatographic behavior for new derivatives. The integration of BMC with QSRR thus represents a rational dual approach for evaluating passive gastrointestinal absorption potential and interpreting structure-retention relationships within a single experimental framework (14).

Considering the biological potential of naproxen-based thiourea derivatives and the need to assess their oral absorption characteristics, this study was focused on evaluating passive gastrointestinal permeation using a BMC model as a biomimetic in vitro surrogate. Additionally, a QSRR analysis was performed to elucidate structural determinants of retention within the BMC system. It should be noted that the present study examines a limited number of compounds belonging to a previously synthesized series of naproxen-based thiourea derivatives. The seven molecules included in this work were selected as representative members of the main structural subgroups within that series, based on previously obtained physicochemical data, particularly lipophilicity measurements and PAMPA permeability results. Although the relatively small dataset represents a limitation from a statistical standpoint, the selected compounds cover the principal structural variations and the range of permeability-related properties observed in the broader series.

MATERIALS AND METHODS

Chemicals

Brij 35 and HPLC grade acetonitrile were purchased from Sigma-Aldrich Chemie Gmbh (Steinheim, Germany), while disodium hydrogen phosphate (Na2HPO4) and o-phosphoric acid (85%) were from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) was acquired from Fisher (Loughborough, UK). Deionized water was prepared using a TKA water-purification system (Niederelbert, Germany).

Tested thiourea derivatives of naproxen (compounds 2-7 and 11) were synthesized as previously described (5,6). Their chemical structures are presented in Figure 2. The numbering of the compounds corresponds to the previously reported synthetic series, and only selected derivatives (compounds 2-7 and 11) were included in the present study. Test solutions (0.01 mg/mL) were prepared by diluting stock solutions of the tested compounds in DMSO (5 mM) with the mobile phase to obtain the desired concentration.

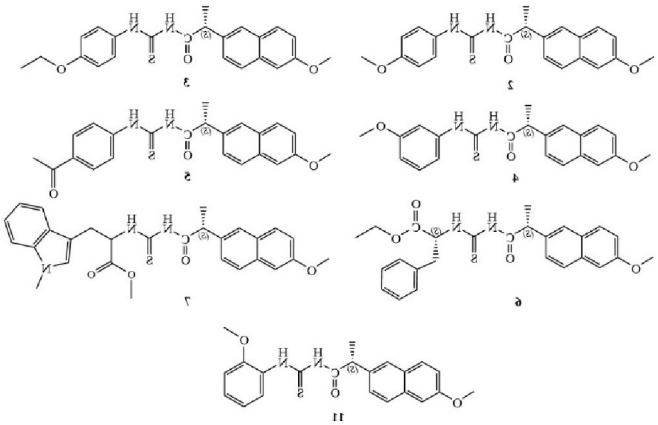


Figure 2. Chemical structures of tested compounds

BMC Analysis

The chromatographic analysis was performed using an HPLC chromatograph (Agilent Technologies 1200 Series Systems, Santa Clara, CA, USA) equipped with a binary pump, manual injector (injection volume 20 µl), and DAD detector. Zorbax Extend-C18 column (150 × 4.6 mm; 5 µm particle size) was used in this study. The mobile phase consisted of acetonitrile and 40 mM polyoxyethylene lauryl ether (Brij 35) dissolved in 7 mM solution of Na_2HPO_4 (30:70 v/v). Previous research has demonstrated that a 0.04 mol/L Brij 35 solution can be combined with up to 30% acetonitrile while still maintaining the formation of stable micelles (15). The pH value of the mobile phase was adjusted to 5.5 by the addition of *o*-phosphoric. The column temperature was set to 36.5°C, the mobile phase flow rate to 1 ml/min, and the detection wavelength to 280 nm for the synthesized compounds and 230 nm for DMSO. Corresponding retention factors (k) were calculated according to the following equation:

$$k = \frac{t_R - t_R(0)}{t_R(0)} \quad (1)$$

where t_R is the retention time of the tested compounds and $t_R(0)$ corresponds to the dead time determined using DMSO as a non-retained marker (10,12,16).

QSRR analysis

QSRR analysis was conducted to elucidate the relationship between chromatographic retention and molecular structure of the investigated compounds. Specifically, correlations were tested between the experimentally determined retention factors (k) obtained from the BMC chromatographic system, used as dependent variables, and a set of selected geometric, thermodynamic, physicochemical, and electronic molecular descriptors serving as independent variables. For QSRR model development, retention data for seven compounds analyzed within the BMC system were utilized.

Prior to descriptor calculation, the molecular structures of all investigated compounds were subjected to geometry optimization. Initial optimization was carried out using the semi-empirical PM3 method, followed by further refinement employing the Hartree-Fock method with the 3-21G basis set to obtain energetically stable conformations (17). A comprehensive set of molecular descriptors was subsequently calculated using the alvaDesc software package (18). An intercorrelation analysis was subsequently conducted to obtain suitable subsets of molecular descriptors for the QSRR study. Descriptor pairs exhibiting intercorrelation coefficients greater than 0.95 were evaluated, and in each case, the descriptor showing a stronger relationship with the dependent variable (retention factor k) was retained for further analysis.

Statistical analysis and model construction were performed using STATISTICA software (19). Descriptor selection for the multiple linear regression model (MLR(k)) was achieved through the combined application of Feature Selection and Variable Screening (FSVS) and the forward stepwise Multiple Linear Regression (MLR) approach. During the forward stepwise MLR procedure, the inclusion and exclusion criteria were set to F-to-enter = 9 and F-to-remove = 4, respectively, while in the FSVS method the parameter k was set to 10. Given the small number of analyzed compounds, which represents a limitation of the present study, the QSRR analysis was intentionally designed as exploratory and restricted to the simplest statistical framework. Accordingly, only a single statistically significant descriptor was included in the model in order to reduce the risk of overfitting and to enable identification of the dominant structural factor governing retention behavior. Consequently, the final QSRR model was developed using the *Mor20u* descriptor without dividing the dataset into training and test subsets.

Due to the limited dataset size (n = 7), external validation procedures such as dataset splitting or cross-validation would not yield statistically reliable estimates of predictive performance. Therefore, the model should be interpreted strictly as

an exploratory structure-retention relationship rather than a predictive QSRR model.

RESULTS AND DISCUSSION

Retention behavior of the tested compounds in the BMC system

BMC was applied to a series of seven thiourea derivatives of naproxen. The experiments were carried out using Brij 35, a nonionic surfactant commonly employed in BMC studies. Chromatographic conditions, including pH, temperature, and ionic strength, were adjusted to biomimetic conditions mimicking biological membranes. Under these conditions, analyte retention is primarily governed by specific interactions with both the modified stationary phase and the micellar pseudophase in the mobile phase (9). Consequently, the elution process reflects a complex interplay between micelles, the stationary phase, and the aqueous medium (20). A critical aspect of method optimization involves establishing an appropriate ratio between the organic modifier and the aqueous phase. Previous investigations have demonstrated that micelle formation is maintained in systems containing 40 mM Brij 35 with up to 30% (v/v) acetonitrile (12). Chromatographic behavior was evaluated using retention time (t_R), retention factor (k), and logarithmic retention factor (log$k$), with the corresponding results summarized in Table 1.

Table 1. Retention factors of the tested compounds in the BMC system.

Compound	t_R(s)	k	log$k$
2	37.24	30.03	1.48
3	35.54	28.62	1.46
4	42.06	34.05	1.53
5	27.57	21.98	1.34
6	57.90	47.25	1.67
7	43.94	35.62	1.55
11	37.90	30.58	1.49

The obtained results revealed that the retention factor (k) values varied between 21.98 and 47.25. The highest retention was observed for compounds **6** (phenylalanine ethyl ester derivative) and **7** (N-methyl tryptophan methyl ester derivative), indicating that thiourea derivatives containing esterified aromatic amino acids are likely to possess enhanced potential for passive gastrointestinal absorption. In contrast, the lowest k value was recorded for derivative **5**, corresponding to the *p*-aminoacetophenone derivative, suggesting a reduced absorption potential for naproxen thiourea derivatives incorporating aromatic amines with a carbonyl functionality. These findings are consistent with our previously published results obtained using the PAMPA assay, which demonstrated superior passive gastrointestinal permeability for aromatic amino acid ester derivatives (21). In contrast, derivative **5**, which in the present study exhibited the lowest predicted potential for passive gastrointestinal absorption, demonstrated moderate permeability across an artificial membrane in a previous PAMPA assay (21).

It should be emphasized that BMC retention represents an indirect physicochemical surrogate reflecting solute partitioning in a biomimetic micellar environment and cannot be considered equivalent to experimentally measured permeability. Consequently, the obtained results should be interpreted as complementary indicators of passive diffusion potential rather than direct measurements of gastrointestinal absorption. It should also be noted that oral absorption is a multifactorial process influenced not only by passive membrane permeability but also by ionization state, dissolution kinetics, intestinal transporters, metabolic stability, and efflux mechanisms. Therefore, chromatographic retention parameters should be interpreted within this broader pharmacokinetic context.

QSRR analysis

Given the limited number of compounds, the simplest statistical model (MLR) was applied, as the aim of the QSRR study was to gain an initial understanding of the key factor governing their retention behavior. Relevant molecular descriptors for the MLR(k) model were identified through a two-stage approach involving FSVS and subsequent forward stepwise MLR. The FSVS procedure selected the *VE2sign_A* descriptor. However, it was not possible to construct a valid MLR(k) model with this descriptor. Using the MLR forward stepwise method, the selected descriptor was *Mor20u*, which yielded a valid model. This model is characterized by high R and R^2 values of 0.977 and 0.955, respectively. The resulting MLR(k) model equation is as follows:

$$\log k = -25.5626 + 18.0194 \times Mor20u \quad (2)$$

Although the obtained correlation coefficients (R and R^2) indicate a strong relationship between retention and the selected descriptor, these values should be interpreted with caution due to the limited dataset size. In small datasets, high correlation coefficients may reflect dataset-specific relationships rather than true predictive performance. Therefore, the presented regression should be considered an exploratory structure-retention relationship within this compound series rather than a validated predictive QSRR model.

Statistical analysis showed that the *Mor20u* was identified as the descriptor most strongly associated with retention behavior in the BMC system. *Mor20u* belongs to the 3D-MoRSE group of molecular descriptors (22), which encode informations related to atomic properties, including polarizability, and their three-dimensional distribution within the molecule. However, it should be emphasized that 3D-MoRSE descriptors are abstract numerical representations and their mechanistic interpretation is not straightforward. (23-26). Studies have shown that the presence of aromatic systems and unsaturated bonds increases the values of these descriptors (26). Since 3D-MoRSE descriptors take into account the three-dimensional arrangement of atoms in a molecule and do not depend on molecular size, they can be applied to a broad range of structures (23). The *Mor20u*

descriptor is generally associated with molecular polarizability and three-dimensional distribution of atomic properties (27). In this context, the observed relationship between *Mor20u* and retention should be interpreted as a statistical correlation rather than a direct mechanistic explanation of solute-micelle or solute-stationary phase interactions. The highest descriptor values were recorded for compounds **6** (phenylalanine ethyl ester derivative) and **7** (N-methyl tryptophan methyl ester derivative), indicating that high *Mor20u* values are characteristic of aromatic amino acid ester derivatives. Conversely, the lowest descriptor value (2.76) within the examined set was observed for compound **5** (*p*-aminoacetophenone derivative). These observations may indicate that esterified aromatic amino acid derivatives tend to exhibit higher retention within this compound series. From a physicochemical perspective, increased aromatic surface area and molecular polarizability may enhance hydrophobic and dispersive interactions with both the C18 stationary phase and the hydrophobic core of micelles, which can contribute to increased retention. Aromatic substituents may also facilitate π-π and van der Waals interactions within the chromatographic system. It should be noted that increasing lipophilicity or molecular polarizability, although potentially beneficial for passive membrane permeability, may be associated with trade-offs such as reduced aqueous solubility, altered distribution, or increased risk of off-target interactions. Therefore, optimization of absorption-related properties should be considered within a broader pharmacokinetic and safety framework (28,29).

However, these interpretations remain qualitative and should be considered as plausible explanations rather than definitive mechanistic conclusions. Due to the limited size of the dataset, the developed QSRR model should be considered exploratory rather than predictive. Additional studies involving a larger and more structurally diverse set of compounds will be required to confirm the robustness and general applicability of the identified relationship.

CONCLUSIONS

In this study, BMC proved to be an effective tool for evaluating the retention behavior and passive absorption-related properties of naproxen-based thiourea derivatives under biomimetic conditions mimicking biological membranes. The obtained results indicated that retention in the BMC system is strongly influenced by molecular structure, particularly the presence of esterified aromatic amino acid moieties, which were associated with higher retention factors and, consequently, a greater potential for passive gastrointestinal absorption. In contrast, incorporation of aromatic amines bearing a carbonyl group resulted in lower retention and reduced absorption potential. The QSRR analysis indicated that the *Mor20u* descriptor was selected as a key variable in the MLR(*k*) model. This descriptor effectively described the relationship between molecular structure and chromatographic retention, emphasizing the importance of molecular polarizability and aromatic character. Taken together, results demonstrate that the combined application of BMC and QSRR represents a powerful and complementary approach for the early assessment of biopharmaceutical properties and may provide preliminary insight that could support future design considerations, which require validation on larger datasets and through complementary experimental approaches.

FUNDING

This research was supported by the Science Fund of the Republic of Serbia, 7739840, *Utilization of interplay between inflammation and cancer in the development of compounds with anticancer activity - INFCANPLAY*. The research was also supported by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, through Grant Agreements with the University of Belgrade - Faculty of Pharmacy (No 451-03-136/2025-03/200161 and No 451-03-137/2025-03/200161) and with Faculty of Medical Sciences, University of Kragujevac No 451-03-34/2026-03/200111, as well as by the Faculty of Medical Sciences, University of Kragujevac (Junior Project 11/20).

ACKNOWLEDGMENTS

Not applicable.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest.

REFERENCES

1. Angiolillo DJ, Weisman SM. Clinical pharmacology and therapeutic use of naproxen. Am J Cardiovasc Drugs. 2017;17(2):97-107.
2. Stoev SN, Gueorguiev SR, Madzharov VG, Lebanova HV. Naproxen in pain and inflammation - a review. Int J Pharm Phytopharmacol Res. 2021;11(1):142-8.
3. Curini M, Epifano F, Fiorito S, Piacente S. Thiourea derivatives: synthesis, biological properties and therapeutic potential. Mini Rev Med Chem. 2012;12(8):776-95.
4. Kumar S, Singh I, Kamboj S, Thakur AS. Thiourea-based anticancer agents: synthesis and biological evaluation. Eur J Med Chem. 2018;157:1137-51.
5. Nedeljković N, Dobričić V, Bošković J, Vesović M, Bradić J, Anđić M et al. Synthesis and investigation of anti-inflammatory activity of new thiourea derivatives of naproxen. Pharmaceuticals. 2023;16(5):666.
6. Nedeljković N, Nikolić M, Čanović P, Zarić M, Živković Zarić R, Bošković J et al. Synthesis, characterization, and investigation of anti-inflammatory and cytotoxic activities of novel thiourea derivatives of naproxen. Pharmaceutics. 2023;16(1):1.
7. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Adv Drug Deliv Rev. 2004;56(1):3-26.
8. Varma MV, Ashokraj Y, Dixit R, Tennikova TB. Physicochemical determinants of intestinal absorption. Pharm Res. 2010;27(12):2828-36.
9. Molero-Monfort M, Escuder-Gilabert L, Villanueva-Camañas RM, Sagrado S, Medina-Hernández MJ.

Biopartitioning micellar chromatography: an in vitro technique for predicting human drug absorption. J Chromatogr B. 2001;753(2):225-36.
10. Čudina O, Marković B, Karljiković-Rajić K, Vladimirov S. Biopartitioning micellar chromatography-partition coefficient micelle/water as a potential descriptor for hydrophobicity in prediction of oral drug absorption. Anal Lett. 2012;45(7):677-88.
11. Stępnik KE, Malinowska I. Biopartitioning micellar chromatography and immobilized artificial membrane chromatography for predicting blood-brain barrier penetration of phenols. J Chromatogr A. 2013;1286:127-36.
12. Dobričić V, Nikolić K, Vladimirov S, Čudina O. Biopartitioning micellar chromatography as a predictive tool for skin and corneal permeability. Eur J Pharm Sci. 2014;56:105-12.
13. Héberger K. Quantitative structure-chromatographic retention relationships. J Chromatogr A. 2007;1158(1-2): 273-305.
14. Kaliszan R, Marini F, Bączek T. Structure-retention relationships in chromatography. J Chromatogr A. 2007;1158(1-2):1-26.
15. Dobričić V, Nikolic K, Vladimirov S, Čudina O. Biopartitioning micellar chromatography as a predictive tool for skin and corneal permeability of newly synthesized 17 β -carboxamide steroids. Eur J Pharm Sci. 2014;56:105-12.
16. Tsopelas F, Danias P, Pappa A, Tsantili-Kakoulidou A. Biopartitioning micellar chromatography under different conditions: Insight into the retention mechanism and the potential to model biological processes. J Chromatogr A. 2020;1621:461027.
17. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR,et al.Gaussian Inc., Wallingford CT, 2010.
18. Alvascience, alvaDesc (software for molecular descriptors calculation) version 2.0.8, https://www.alvascience.com, 2021.
19. Statistica, version 13; TIBCO Software Inc., Palo Alto, USA, 2018.
20. Arunyanart M, Love LJC. Model for Micellar Effects on Liquid Chromatography Capacity Factors and for Determination of Micelle-Solute Equilibrium Constants. Anal Chem. 1984;56,1557-61.
21. Nedeljković N, Dobričić V, Vesović M, Jelić R, Marković B, Vujić Z, Nikolić M. In Vitro Pharmacokinetic Profiling of Thiourea Derivatives of Naproxen With Anti-Inflammatory and Anticancer Activity. Chem. Biodivers. 2026;23(1):e02219.
22. Molecular Descriptors, QSAR, Chemometrics and Chemoinformatics. Dragon Molecular Descriptor List.2013.
23. Duchowicz PR, Fernández M, Caballero J, Castro EA, FernándezFM. QSAR for non-nucleoside inhibitors of HIV-1 reverse transcriptase. Bioorgan Med Chem. 2006;14:5876-89.
24. Hancock T, Put R, Coomans D, Vander Heyden Y, Everingham Y. A performance comparison of modern statistical techniques for molecular descriptor selection and retention prediction in chromatographic QSRR studies. Chemometr Intell Lab Systems.2005;76:185-96.
25. Todeschini R, Consonni V. Handbook of molecular descriptors. Wiley-VCH, Weinheim. 2000. doi: 10.1002/9783527613106
26. Duchowicz PR, Fernandez M, Caballero J, Castro EA, Fernandez FM. QSAR for non-nucleoside inhibitors of HIV-1 reverse transcriptase. Bioorg Med Chem. 2006;14:5876-89.
27. Xu J, Wang L, Zhang H, Shen X, Liang G. Quantitative structure-property relationships studies on free-radical polymerization chain-transfer constants for styrene. J Appl Polym Sci. 2012;123(1):356-64.
28. Veber DF, Johnson SR, Cheng HY, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002;45(12):2615-23.
29. Azman M, Sabri AH, Anjani QK, Mustaffa MF, Hamid KA. Intestinal Absorption Study: Challenges and Absorption Enhancement Strategies in Improving Oral Drug Delivery. Pharmaceuticals (Basel). 2022;15(8):975.

INFLUENCE OF TYPE 2 DIABETES MELLITUS ON THE CORNEAL ENDOTHELIUM AND CENTRAL CORNEAL THICKNESS AFTER PHACOEMULSIFICATION

Milos Potezica[1], Tatjana Sarenac Vulovic [2,3], Jovana Srejovic[2,3], Katarina Cupic[2,3], Teodora Todorovic[4] and Dusan Todorovic[2,3]

[1]*University of Kragujevac, Faculty of Medical Sciences*
[2]*University of Kragujevac, Faculty of Medical Sciences, Department of Ophthalmology*
[3]*Clinic for Ophthalmology, University Clinical Center Kragujevac, Serbia,*
[4] *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacy*

Received: 11.02.2026.
Accepted: 13.04.2026.

Corresponding author:

Milos Potezica

University of Kragujevac, Faculty of Medical Sciences

E-mail: milospotezica777@gmail.com

UDK:

Eabr 2026; 27(2):173-178
DOI:10.2478/eabr-2026-0018

ABSTRACT

Diabetes mellitus may compromise corneal endothelium and increase central corneal thickness, potentially influencing recovery after phacoemulsification. In this retrospective cohort study, we reviewed 45 patients (76 eyes) who underwent phacoemulsification from November 2023 to May 2024. The diabetes group included 36 eyes of patients with type 2 diabetes mellitus, and control group included 40 eyes of non-diabetic patients. Specular microscopy was performed preoperatively and 1 month postoperatively to assess endothelial cell density, percentage of hexagonal cells, coefficient of variation, and central corneal thickness. Endothelial cell density decreased significantly in diabetes group (2577.25 ± 191.22 to 1899.19 ± 682.59 cells/mm²; $p < 0.001$). In the control group, ECD decreased significantly from 2460.35 ± 307.80 to 1786.18 ± 702.56 cells/mm² ($p < 0.001$). There was no intergroup difference in endothelial cell density preoperatively ($p > 0.05$), whereas difference after cataract surgery was significant ($p < 0.05$). The percentage of hexagonal cells and coefficient of variation did not differ significantly within or between groups ($p > 0.05$). Central corneal thickness was higher in diabetes group both preoperatively (545.61 ± 32.45 vs 522.74 ± 36.49 µm) and postoperatively (556.32 ± 21.07 vs 536.69 ± 36.63 µm), with significant intergroup differences at both time points ($p < 0.05$); within-group changes were not significant ($p > 0.05$). Compared with controls, eyes with type 2 diabetes mellitus showed lower postoperative endothelial cell density and persistently higher central corneal thickness 1 month after phacoemulsification.

Keywords: *Diabetes mellitus, phacoemulsification, corneal endothelium, endothelial cell density, central corneal thickness.*

INTRODUCTION

Diabetes mellitus (DM) reprsents a chronic metabolic disease with a high prevalence and a substantial burden on health-care systems, with a global prevalence exceeding half a billion people older than 20 years, corresponding to roughly 10% of the adult population (1). The pathophysiological basis of DM involves the combined effects of chronic hyperglycemia, oxidative stress, activation of the polyol pathway with intracellular sorbitol accumulation, and non-enzymatic protein glycation, which can lead to microcirculatory disturbances, structural damage, and impaired homeostasis in various tissues, including the eye (2,3).

The impact of DM on the posterior segment and the development of diabetic retinopathy is well established (1,2). In recent decades, however, the effects of DM on the anterior segment have received increasing attention (1,3). On the corneal surface and in the epithelial layer, changes consistent with diabetic keratopathy are often observed, including thickening and multilamination of the epithelial basement membrane, reduced corneal sensitivity, and delayed epithelial healing (4,5). Chronic hyperglycemia activates the sorbitol-aldose reductase pathway, and the accumulation of sorbitol and fructose disturbs Na+/K+-ATPase activity in the endothelial cell membrane, contributing to reduced barrier function (6). In DM, corneal endothelial cells may exhibit decreased endothelial cell density (ECD), increased polymegatism and pleomorphism, and a reduced percentage of hexagonal cells, indicating compromised morphological stability and a reduced functional reserve. Given that cataract in patients with DM occurs earlier and progresses more rapidly than in the general population, these endothelial alterations become particularly relevant (7). Multiple studies report lower ECD and increased central corneal thickness (CCT) in patients with type 2 DM (DM2) compared with healthy individuals, suggesting impaired endothelial stability (8). At the same time, cataract surgery is among the most frequently performed surgical procedures in medicine (6). Together, these factors may influence postoperative visual outcomes and satisfaction in patients with DM after cataract surgery.

Corneal endothelial status is particularly important in the perioperative period of phacoemulsification. Although phacoemulsification is the gold standard in cataract surgery, it exposes the corneal endothelium to multimodal stress, including irrigation fluids and ultrasound energy with acoustic cavitation, which may induce oxidative stress (10,11). Because the corneal endothelium in DM2 is morphologically weakened and has a lower functional reserve, the consequences of intraoperative stress may be more pronounced, with greater early postoperative endothelial cell loss (4,10). Patients with DM may also develop more pronounced postoperative corneal thickening (increased CCT), especially early after phacoemulsification, compared with non-diabetic patients (12). These findings suggest that DM may prolong corneal recovery and, consequently, delay visual recovery after phacoemulsification (9-12).

The primary aim of this study was to evaluate the effect of DM on central corneal thickness and corneal endothelial changes after phacoemulsification.

MATERIALS AND METHODS

Study design and participants

This retrospective cohort study was conducted from November 2023 to May 2024 and included 76 eyes: 36 eyes of patients with type 2 diabetes mellitus and 40 eyes of non-diabetic participants (control group). The eye was used as the unit of analysis. The follow-up time point was 1 month after cataract surgery.

Inclusion criteria were age >18 years, preoperative ECD ≥1500 cells/mm², and the presence of a developed cataract. Exclusion criteria included previous intraocular trauma or surgical interventions, conditions with a potential major impact on the corneal endothelium (corneal dystrophies, uveitis, glaucoma), and high myopia or hyperopia greater than 5 diopters. Data on sex, age, comorbidities, and patient habits were collected from patient history and medical documentation.

Ethics and informed consent

The study was approved by the Ethics Committee of Specijalna bolnica Sveti Vid (Approval No. 19) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients, and all data were analyzed in anonymized form.

Preoperative assessment

All patients underwent a complete preoperative ophthalmic examination, including best-corrected visual acuity (BCVA) assessment using Snellen charts, intraocular pressure (IOP) measurement by applanation tonometry (Haag-Streit), slit-lamp examination of the anterior segment, dilated fundus examination, intraocular lens (IOL) power calculation (optical biometry), and ocular ultrasonography. Corneal endothelial parameters were obtained by specular microscopy (Tomey EM-3000) (Figure 1). The analyzed parameters were ECD, percentage of hexagonal cells, coefficient of variation, and CCT.

Figure 1. Tomey EM-3000 specular microscope

Surgical procedure

All surgeries were performed by three experienced cataract surgeons under topical anesthesia (tetracaine). The phacoemulsification machine used in all procedures was Stellaris (Bausch & Lomb, Laval, Quebec, Canada). Maximal preoperative mydriasis was achieved with topical phenylephrine and homatropine. Two 1.5 mm paracenteses were created at 2 and 10 o'clock, and the anterior chamber was filled with a cohesive viscoelastic (ProVisc®, Alcon Laboratories, Inc., Fort Worth, TX, USA). A 2.2 mm clear corneal incision was made at 12 o'clock. Continuous curvilinear capsulorhexis, hydrodissection, and nucleus rotation were performed. The nucleus was fragmented using the divide-and-conquer technique. Residual cortex was removed using bimanual irrigation and aspiration. After filling the capsular bag with cohesive viscoelastic, an IOL was implanted. Viscoelastic was aspirated, and intracameral cefuroxime (1 mg/0.1 mL in balanced salt solution) was injected. Corneal incisions were hydrated using balanced salt solution. At the conclusion of surgery, standard topical medication was administered according to the institutional protocol. Postoperatively, patients were prescribed topical dexamethasone/tobramycin five times daily and nepafenac three times daily, followed by gradual tapering according to the standard postoperative regimen.

Postoperative assessment

One month after phacoemulsification, all patients underwent a complete ophthalmic examination with repeated corneal measurements (ECD, percentage of hexagonal cells, coefficient of variation, and CCT).

Sample size estimation

The effect size used for sample size estimation was derived from the study by El-Saied et al. (2023) (20), which reported a statistically significant postoperative difference in endothelial cell density between diabetic and non-diabetic eyes at 1 month after phacoemulsification.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics (version 27.0). Continuous variables were summarized as mean ± standard deviation (SD) for normally distributed data or median (interquartile range) for non-normally distributed data. Categorical variables were presented as frequencies and percentages. Normality of distribution was assessed using the Shapiro–Wilk test. Pre- and postoperative comparisons within groups were performed using the paired Student's t-test for normally distributed variables and the Wilcoxon signed-rank test for non-normally distributed variables. Between-group comparisons (type 2 diabetes mellitus vs control group) were conducted using the independent-samples t-test or the Mann–Whitney U test, as appropriate. Effect sizes were calculated using Cohen's d for parametric tests and r for non-parametric tests. A two-sided p value < 0.05 was considered statistically significant.

RESULTS

A total of 45 patients (76 eyes) were included. The diabetes group comprised 22 patients (36 eyes), and the control group comprised 23 patients (40 eyes). In the overall sample, there were 24 women (53.3%) and 21 men (46.7%), with no statistically significant difference in sex distribution between the groups. Mean age was 67.4 ± 10.1 years in the diabetes group and 65.8 ± 12.6 years in the control group, without a statistically significant difference ($p > 0.05$) (Table 1).

In the diabetes group, preoperative ECD was 2577.25 ± 191.22 cells/mm² and decreased to 1899.19 ± 682.59 cells/mm² at 1 month postoperatively, representing a highly significant change ($p < 0.001$). In the control group, ECD was 2460.35 ± 307.80 cells/mm² preoperatively and 1786.18 ± 702.56 cells/mm² postoperatively, representing a statistically significant reduction ($p < 0.001$). Intergroup comparison revealed no statistically significant difference in preoperative ECD ($p > 0.05$), but a significant postoperative difference was found ($p < 0.05$).

In both groups, cataract surgery did not result in a statistically significant change in the percentage of hexagonal cells ($p > 0.05$). In the diabetes group, the percentage of hexagonal cells decreased from 43.2 ± 5.4% preoperatively to 39.4 ± 8.2% postoperatively, while in the control group it decreased from 45.1 ± 7.8% to 42.2 ± 7.6%. Intergroup comparisons of hexagonality before and after surgery showed no statistically significant differences.

The coefficient of variation increased from 38.9 ± 5.5% preoperatively to 40.7 ± 10.2% postoperatively in the diabetes group, without a statistically significant difference ($p > 0.05$). In the control group, the coefficient of variation was 35.6 ± 3.3% before surgery and 37.5 ± 6.1% after surgery, also without a statistically significant difference ($p > 0.05$).

Central corneal thickness in the diabetes group was 545.61 ± 32.45 µm preoperatively and 556.32 ± 21.07 µm postoperatively, without a statistically significant within-group change ($p > 0.05$). In controls, CCT was 522.74 ± 36.49 µm preoperatively and 536.69 ± 36.63 µm postoperatively, also without a statistically significant within-group change. However, CCT differed significantly between the diabetes and control groups both before and after phacoemulsification ($p < 0.05$).

Table 1. Age and sex distribution

Variable	DM group (n = 36 eyes / 22 patients)	Control group (n = 40 eyes / 23 patients)
Age (years), mean ± SD	67.4 ± 10.1	65.8 ± 12.6
Sex (patients), n (%)		
Male	11 (50.0%)	10 (43.5%)
Female	11 (50.0%)	13 (56.5%)

Table 2. Preoperative and postoperative corneal parameters in the diabetes and control groups

Parameter	DM Pre-op (Mean ± SD)	DM 1 Month (Mean ± SD)	p (within DM)	Control Pre-op (Mean ± SD)	Control 1 Month (Mean ± SD)	p (within Control)	p (between groups)
ECD (cells/mm²)	2577.25 ± 191.22	1899.19 ± 682.59	<0.001	2460.35 ± 307.80	1786.18 ± 702.56	< 0.001	Pre-op: >0.05 Post-op: <0.05
HEX (%)	43.2 ± 5.4	39.4 ± 8.2	>0.05	45.1 ± 7.8	42.2 ± 7.6	>0.05	>0.05
CV (%)	38.9 ± 5.5	40.7 ± 10.2	>0.05	35.6 ± 3.3	37.5 ± 6.1	>0.05	>0.05
CCT (µm)	545.61 ± 32.45	556.32 ± 21.07	>0.05	522.74 ± 36.49	536.69 ± 36.63	>0.05	Pre-op: <0.05 Post-op: <0.05

DISCUSSION

Diabetes mellitus is a systemic disease that affects multiple organs, and ocular involvement is common. In addition to diabetic retinopathy, patients may develop cataract, neovascular glaucoma, and other complications that can threaten vision. Corneal involvement in diabetes, including diabetic keratopathy and endothelial dysfunction, may reduce the functional reserve of the corneal endothelium, thereby increasing susceptibility to postoperative stromal edema, dry eye, decreased corneal sensitivity, and delayed visual recovery (1).

Phacoemulsification has become the standard technique for cataract surgery over the past several decades. The procedure is based on a phaco probe containing a piezoelectric crystal that vibrates at ultrasonic frequencies, generating cavitation within the crystalline lens and allowing its fragmentation and removal. This technique has significantly improved surgical precision, reduced incision size, and enhanced postoperative visual recovery. It has also lowered the incidence of many intraoperative and postoperative complications. Nevertheless, complications may still occur (13). Corneal edema remains one of the most common postoperative complications, primarily due to endothelial cell stress or loss during surgery. In patients with diabetes mellitus, chronic hyperglycemia further increases endothelial vulnerability.

Oxidative stress and metabolic imbalance impair endothelial cell function, predisposing these patients to a more pronounced postoperative response leading to dysfunction of corneal endothelial cells. Damage to these cells results in corneal edema, clinically manifested by decreased visual acuity.

In the present study, eyes of patients with type 2 diabetes mellitus showed a statistically significant reduction in ECD one month after phacoemulsification. A statistically significant endothelial cell loss was observed in both groups; however, however, postoperative endothelial parameters were less favorable in the diabetes group. In addition, CCT was higher in the diabetes group both preoperatively and postoperatively, and intergroup differences were significant at both time points. Earlier studies have suggested that increased CCT may represent an early manifestation of diabetes-related corneal involvement (15,16). By reducing Na^+/K^+-ATPase activity in the endothelial layer, diabetes mellitus promotes corneal hydration. In addition, hyperglycemia increases aldose reductase activity, an enzyme responsible for sorbitol synthesis. Accumulated sorbitol acts as an osmotic agent, drawing water into the cornea and contributing to increased thickness (16).

These findings support the recommendation that preoperative assessment of corneal endothelial status, including specular microscopy and CCT measurement, should be part of routine evaluation in diabetic patients scheduled for phacoemulsification (17). Endothelial-protective surgical strategies - appropriate viscoelastic use, minimizing ultrasound time and energy, maintaining anterior chamber stability, and gentle cortical manipulation - may help limit endothelial loss. Postoperatively, closer follow-up and adequate anti-inflammatory therapy may be warranted in patients with diabetes (6).

Although diabetes mellitus may increase the risk of corneal endothelial damage, with careful preoperative assessment and contemporary surgical technique, phacoemulsification can remain a safe and effective procedure in this population (19). Phacoemulsification remains the gold standard in cataract surgery; however, special caution is required in patients with diabetes mellitus due to existing structural and functional alterations of the corneal endothelium. Numerous clinical and experimental studies have demonstrated that diabetic patients exhibit reduced endothelial cell density, increased polymegatism and pleomorphism, as well as diminished endothelial functional reserve, making them more susceptible to postoperative complications (8).

Our results further indicate that diabetes mellitus had a significant impact on central corneal thickness. Phacoemulsification in diabetic patients was associated with a more pronounced endothelial cell loss and an increase in central corneal thickness at 1 month postoperatively compared to non-diabetic patients, particularly in cases of long-standing diabetes and poor glycemic control. Mechanical, thermal, and oxidative stress during surgery additionally burden the already compromised endothelium, thereby increasing the risk of postoperative corneal edema and prolonged visual recovery (19).

Our findings are consistent with several previously published studies that evaluated corneal endothelial changes in diabetic patients undergoing phacoemulsification. Chaurasia et al. (7) reported less favorable postoperative endothelial parameters in diabetic eyes compared with non-diabetic controls after cataract surgery, which is in line with our results. Similarly, a recent meta-analysis by Yang et al. (8) demonstrated that diabetes mellitus is associated with increased endothelial vulnerability and a higher susceptibility to postoperative endothelial damage. In addition, studies investigating central corneal thickness have shown that diabetic patients tend to exhibit thicker corneas both before and after surgery, likely reflecting impaired endothelial pump function (12,17). Our results support these observations, as we also observed significantly higher CCT values in the diabetes group at both time points. Taken together, these findings suggest that diabetes-related endothelial alterations may contribute to less favorable postoperative corneal status after phacoemulsification.

However, by applying modern surgical techniques, careful intraoperative management, adequate selection of viscoelastic agents, and reduction of ultrasound time and energy, the negative impact of phacoemulsification on the corneal endothelium in diabetic patients can be significantly minimized. Preoperative endothelial assessment using specular microscopy, together with appropriate systemic control of diabetes mellitus, remains essential for achieving optimal surgical outcomes.

Although phacoemulsification in patients with diabetes mellitus carries an increased risk of endothelial damage, with proper patient selection and an individualized surgical approach, this method remains safe and effective in the treatment of cataracts in this population.

CONCLUSIONS

Eyes of patients with type 2 diabetes mellitus showed lower postoperative endothelial cell density 1 month after phacoemulsification compared with non-diabetic controls. Central corneal thickness was higher in the diabetes group both before and after surgery, while changes in hexagonality and the coefficient of variation were not statistically significant. Routine preoperative endothelial evaluation and endothelial-protective surgical technique may be particularly important in diabetic patients undergoing phacoemulsification.

STATEMENT OF HUMAN RIGHTS

This study was conducted in accordance with the ethical standards of the institutional research committee (Ethics Committee of Eye Hospital „Sveti Vid“, Approval No. 19) and with the 1964 Helsinki Declaration and its later amendments.

STATEMENT OF INFORMED CONSENT

Written informed consent was obtained from all patients, and all data were analyzed in anonymized form.

ACKNOWLEDGMENTS

The authors have no acknowledgments to declare.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Zarei-Ghanavati S, Hadi Y, Habibi A, Ashraf Khorasani M, Yoo SH. Cataract and diabetes: review of the literature. J Cataract Refract Surg. 2024;50(12):1275-83.
2. Mishra D, Kashyap A, Srivastav T, Yadav A, Pandey S, Majhi MM, et al. Enzymatic and biochemical properties of lens in age-related cataract versus diabetic cataract: A narrative review. Indian J Ophthalmol. 2023;71(7):2379-84.

3. Kiziltoprak H, Tekin K, Inanc M, Goker YS. Cataract in diabetes mellitus. World J Diabetes. 2019;10(3):140-53.
4. Chowdhury B, Bhadra S, Mittal P, Shyam K. Corneal endothelial morphology and central corneal thickness in type 2 diabetes mellitus patients. Indian J Ophthalmol. 2021;69(7):1718-24.
5. Yeung A, Dwarakanathan S. Diabetic keratopathy. Dis Mon. 2021;67(5):101135.
6. Yu FX, Lee PSY, Yang L, Gao N, Zhang Y, Ljubimov AV, et al. The impact of sensory neuropathy and inflammation on epithelial function in diabetic keratopathy. Prog Retin Eye Res. 2022;88:101039.
7. Chaurasia RK, Khasnavis A, Mittal J. Comparison of corneal endothelial changes following phacoemulsification in diabetic and non-diabetic patients. Indian J Ophthalmol. 2022;70(4):1186-92.
8. Yang Y, Chai H, Ding Z, Tang C, Liang Y, Li Y, et al. Meta-analysis of corneal endothelial changes after phacoemulsification in diabetic patients. BMC Ophthalmol. 2023;23:244.
9. Sato T. Corneal endothelial changes after phacoemulsification using the eight-chop technique in diabetic eyes. J Pers Med. 2025;15(5):209.
10. Opala A, Kołodziejski Ł, Grabska-Liberek I. Impact of well-controlled type 2 diabetes on corneal endothelium following cataract surgery. J Clin Med. 2025;14(10):3603.
11. Chen K, Xu WY, Sun SS, Zhou HW. Corneal endothelial cells and acoustic cavitation in phacoemulsification. World J Clin Cases. 2023;11(8):1712-20.
12. Antičić-Eichwalder M, Lex S, Sarny S, Schweighofer J, Marić I, El-Shabrawi Y. Effects of type 2 diabetes mellitus and smoking on corneal endothelium and central corneal thickness in cataract patients. Cornea. 2022;41(6):735-42.
13. Younus O, Lockington D. Comparison of phacoemulsification ultrasonic power between the phaco-chop and the divide-and-conquer techniques. Eye (Lond). 2024;38(1):68-76.
14. Keeratidamkerngsakul B, Puangsricharern V, Chokesuwattanaskul S, Pongpirul K, Kittipibul T. Efficacy of the Rho-kinase inhibitor ripasudil in preventing corneal edema after phacoemulsification. Cornea. 2025;44(7): 896-904.
15. Shakya K, Pokharel S, Karki KJ, Pradhananga C, Pokharel RP, Malla OK. Corneal edema after phacoemulsification surgery in diabetic and non-diabetic patients. Nepal J Ophthalmol. 2013;5(2):230-4.
16. Jing Z, Hao J, Sun L, Zhao X, Jia X, Liu Z, et al. Analysis of influencing factors of corneal edema after phacoemulsification surgery in diabetic patients. Cell Mol Biol (Noisy-le-grand). 2023;69(4):164-71.
17. Uzunoglu A, Valenzuela-Fuenzalida JJ, Morales-Calderón K, Aguilar-Aguirre I, Bruna-Mejias A, Nova-Baeza P, et al. The effect of diabetes mellitus on central corneal thickness values: a systematic review and meta-analysis. Int J Mol Sci. 2025;26(17):8695.
18. Das S, Nanaiah SG, Kummelil MK, Nagappa S, Shetty R, Shetty BK. Effect of fluidics on corneal endothelial cell density, central corneal thickness, and central macular thickness after phacoemulsification with torsional ultrasound. Indian J Ophthalmol. 2015;63(8):641-4.
19. Ciorba AL, Roiu G, Abdelhamid AM, Saber S, Cavalu S. Evaluation of the corneal endothelium following cataract surgery in diabetic and non-diabetic patients. Diagnostics (Basel). 2023;13(6):1115.
20. El-saied ENA, Elrahman HMA, El-Halim NEDAH, Abdullah AS, Lamey NM. Evaluation of corneal endothelial cell changes in diabetic patients after phacoemulsification surgery. Al-Azhar Int Med J. 2023;4(3):128-133.

PREDICTORS OF ADVERSE DRUG REACTIONS TO RESERVE ANTIBIOTICS IN HOSPITALIZED PATIENTS: A TERTIARY CARE STUDY

Nemanja Z. Petrovic[1,2], Mirjana Milojevic Corbic[2] and Slobodan M. Jankovic[1,2]

[1] *University of Kragujevac, Faculty of Medical Sciences, Department of Pharmacology and Toxicology, Kragujevac, Serbia*
[2] *Department of Clinical Pharmacology, University Clinical Center Kragujevac, Kragujevac, Serbia*

Received: 10.03.2026.
Accepted: 27.03.2026.

Corresponding author:

Nemanja Z. Petrovic

University of Kragujevac,Faculty of Medical Sciences, Department of Pharmacology and Toxicology, Svetozara Markovica 69, 34000 Kragujevac, Serbia

E-mail: dr.nemanja.z.petrovic@gmail.com

UDK:

Eabr 2026; 27(2):179-186
DOI:10.2478/eabr-2026-0014

ABSTRACT

Reserve antibiotics are frequently used in hospitalized patients with severe infections, particularly in tertiary healthcare institutions. Although these agents are essential for the treatment of multidrug-resistant infections, their use may be associated with an increased risk of adverse drug reactions(ADRs). Identification of clinical and laboratory predictors of ADRs may contribute to safer and more rational antibiotic therapy. To determine the frequency of ADRs during treatment with reserve antibiotics in hospitalized patients at the University Clinical Center Kragujevac(UCCKg) and to identify factors associated with their occurrence. This observational study had a prospective–retrospective design and included spontaneous reporting of ADRs to reserve antibiotics by clinical pharmacologists during regular consultations with patients receiving these drugs. The study was conducted in all units of the UCCKg among patients for whom a consultation with a clinical pharmacologist had been requested by the attending physician between January 2022 and December 2023. Data were collected from the consultation registry archive and the electronic health information system of the UCCKg. Among patients receiving reserve antibiotics who were consulted by clinical pharmacologist, 52 developed ADRs. Significant factors associated with their occurrence were chronic renal insufficiency(adjusted OR 3.36), heart failure(adjusted OR 0.26), and peptic ulcer disease(adjusted OR 0.27). Reserve antibiotics are essential in the treatment of severe infections and are frequently used in tertiary healthcare institutions. Their use is associated with a certain incidence of ADRs, particularly in patients with renal failure or prolonged severe infections. These factors should be considered during treatment planning to prevent unfavorable outcomes.

Keywords: *Adverse drug reactions, reserve antibiotics, hospitalized patients, risk factors, pharmacovigilance.*

INTRODUCTION

Adverse drug reactions (ADRs) are responses to a medicinal product that are noxious and unintended, and that occur at doses normally used in humans or arise from the use of a medicinal product within or outside the terms of the marketing authorization, including medication errors, misuse, abuse, and off-label use (1,2). ADRs are a serious public health issue due to their potential to greatly increase morbidity, death, and financial burden (3). Up to 0.1–1% of patients using systemic drugs experience ADRs, which are a global health concern. These are expensive for the healthcare systems and have an effect on patient care and health-related quality of life (4). Research indicates that hospital admissions were caused by 5–10% of ADRs. Hospital admissions for ADRs were 5.5% in underdeveloped countries and 6.3% in wealthy countries. According to an Indian study, the prevalence of ADRs was 3.7%, and 0.7% of hospitalizations were caused by ADRs (5).

Based on patient characteristics (gender, age, weight, creatinine clearance, and number of comorbidities) and drug administration (dosage, administration method, number of concurrent drugs), high-risk agents should be properly monitored. Extremes in age, gender, the use of many medications, a medical condition, a history of allergies or ADRs, genetics, high dosages, and numerous other factors can all raise the risk of ADRs. In some groups, particularly the elderly, stopping medication or altering dosage may have a significant role in the development of ADRs (6–9). Effective healthcare management and patient safety depend on an understanding of ADRs (10). Hospitalized patients are particularly vulnerable to ADRs due to the severity of their underlying diseases, polypharmacy, and the frequent use of high-risk medications. Antibiotics are among the most commonly prescribed drugs in hospital settings, and their use is often associated with a considerable number of adverse reactions (11). In addition to allergic reactions, antibiotics may cause gastrointestinal disturbances, hepatotoxicity, nephrotoxicity, hematological disorders, and neurological complications (12,13).

Reserve antibiotics, including carbapenems, glycopeptides, polymyxins, and oxazolidinones, are primarily used for the treatment of severe infections caused by multidrug-resistant microorganisms. Although these drugs are highly effective, they are also associated with a higher risk of ADRs, particularly in critically ill and hospitalized patients. Their toxicity profile and the complex clinical conditions of treated patients make pharmacovigilance and careful monitoring essential (14,15). In tertiary healthcare institutions, the use of reserve antibiotics has increased significantly due to the rising prevalence of antimicrobial resistance (16). Despite their clinical importance, data regarding the frequency and risk factors for ADRs with these drugs remain limited, particularly in hospitalized populations. Therefore, the aim of this study was to determine the frequency of ADRs during treatment with reserve antibiotics in hospitalized patients at the University Clinical Center Kragujevac and to identify factors associated with their occurrence.

MATERIALS AND METHODS

Study design, population, sampling procedure

This study had an observational design with a mixed prospective–retrospective approach and included spontaneous reporting of ADRs to reserve antibiotics by clinical pharmacologists during their routine consultations. The study was conducted among patients who were receiving or had received reserve antibiotics.

The study population consisted of patients hospitalized at the University Clinical Center Kragujevac between January 2022 and December 2023. Cases were defined as hospitalized patients who developed ADRs associated with reserve antibiotics. The control group consisted of patients hospitalized during the same period who were also treated with reserve antibiotics but did not develop ADRs. Controls were matched to cases in a 1:2 ratio.

The inclusion criteria were: hospitalization at the University Clinical Center Kragujevac, treatment with reserve antibiotics, and complete medical documentation. Exclusion criteria included incomplete medical records, confirmed drug overdose, and age under 18 years.

A consecutive convenience sampling method was used for the selection of cases, meaning that all patients with reported ADRs during the study period were included. For each case, potential controls were selected among patients of the same sex and similar age (± 5 years).

The dependent variable was the occurrence of ADRs, determined based on the consultation report of a specialist physician recorded in the patient's medical documentation. ADRs were defined as any harmful and unintended response to a drug that occurs at doses normally used in humans for the purposes of treatment, prevention, diagnosis, or modification of physiological functions, or occurring at any dose during a clinical trial.

Independent variables included length of hospitalization, number of consulted physicians, total number of consultations, and whether the patient had been treated in an intensive care unit. All variables were obtained from patients' medical records.

Potential confounding variables included patient education level, marital status, place of residence (urban or rural), occupation, lifestyle habits (smoking, alcohol consumption, coffee intake), Charlson Comorbidity Index, and specific comorbidities such as chronic renal insufficiency, heart failure, type 2 diabetes mellitus, hyperlipidemia, malignancy, liver disease, and atrial fibrillation. Comorbidities were assessed using the Charlson Comorbidity Index, a validated method for classifying prognostic comorbidity in longitudinal studies (17). These variables were derived from patients' medical records. The primary reason for hospitalization and the medications administered during hospitalization were also recorded.

Data were independently extracted by two researchers from computerized patient medical records stored in the hospital information system "ZIS" (Comtrade, Belgrade). Any discrepancies between the extracted datasets were resolved by consensus in order to ensure accuracy and consistency.

Statistical analysis

The minimum required sample size for the study was calculated using the G*Power software (version 3.1.9.7) (18) based on results from a previously published study (19). The calculation was performed using the following parameters: probability of type I error (α) of 0.05, minimum statistical power of 0.80, odds ratio of 2.6, outcome frequency of 0.26, coefficient of determination (R^2) of 0.40, and normal distribution of variables. Based on these parameters, the calculated minimum sample size was 33 patients in the case group, while the control group was planned to include twice as many participants.

The collected data were numerically coded, tabulated, and subsequently checked for possible errors by the two investigators. Descriptive statistics were used to summarize the data. Continuous variables were presented as mean values with standard deviation when normally distributed, while median and interquartile range were used when data were not normally distributed. Categorical variables were expressed as frequencies, percentages, and relative proportions.

The influence of potential predictors and confounding variables on the occurrence of ADRs was assessed using multivariate binary logistic regression analysis. Prior to applying the regression model, the assumptions for multivariate analysis were verified, including binary outcome variable, independence of observations, absence of multicollinearity among predictors, lack of extreme outliers, and adequate sample size for logistic regression analysis.

The quality and goodness-of-fit of the regression model were evaluated using Cox & Snell R^2, Nagelkerke R^2, and the Hosmer–Lemeshow goodness-of-fit test.

A p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 18.0.

Ethical approval: The study was performed in line with all the relevant national regulations, institutional policies, and in accordance with the tenets of the Helsinki Declaration and has been reviewed and approved by the institutional review board, the Ethics Committee of University Clinical Center Kragujevac [approval number: 01/24-131].

Informed consent statement: Written informed consent was obtained from all individual participants included in the study.

RESULTS

Out of a total of 9,621 clinical pharmacologist consultations during the two-year study period, 156 patients were included in the analysis. Among them, 52 patients experienced an ADR, while 104 patients formed the control group. Overall, 47 patients died, whereas 11 required prolonged hospitalization. Data on the characteristics of the study sample are presented in Tables 1–4, which include demographic and clinical parameters.

Distribution of patient comorbidities are presented in Table 1.

The most frequently prescribed antibiotics were vancomycin, followed by meropenem. Their distribution is shown in Table 2.

The most notable laboratory abnormalities were observed for leukocyte count, serum creatinine, and C-reactive protein (CRP). Mean values are presented in Table 3.

A slightly higher proportion of patients in the total sample had a fatal outcome, while a smaller percentage required prolonged hospitalization. Other relevant characteristics of the study population are presented in Table 4.

Table 5 presents the characteristics of patients according to the study outcome (presence of ADR). No statistically significant difference in sex distribution was observed between the groups. A considerable proportion of patients had diabetes, chronic renal insufficiency, and heart failure.

The relationship between independent variables, potential confounders, and the occurrence of ADRs was examined using multivariate binary logistic regression analysis. A forward conditional stepwise method was applied to construct the regression model including the following predictors: heart failure, peptic ulcer disease, chronic renal insufficiency, age. sex, Charlson comorbidity index, length of hospitalization, leukocyte count, CRP level.

All assumptions for logistic regression analysis were satisfied. There were no extreme outliers, the sample size was adequate, no multicollinearity was detected (variance inflation factor < 2 for all predictors), observations were independent, and residuals followed an approximately normal distribution. Six atypical observations were excluded from the analysis.

The Hosmer–Lemeshow test showed good model fit ($\chi^2 = 1.916$, df = 5, p = 0.861). The Cox & Snell R^2 was 0.118 and the Nagelkerke R^2 was 0.164.

Variables included in the final model are presented in Table 6. Heart failure, peptic ulcer disease, and chronic renal insufficiency were identified as factors associated with the occurrence of ADRs. Heart failure and peptic ulcer disease were identified as protective factors, whereas chronic renal insufficiency was identified as a risk factor for ADRs.

Table 1. Distribution of patient comorbidities (n = 156)

Comorbidity	Frequency (percentage)
Type 2 diabetes mellitus (yes/no)	50/106 (32.1%/67.9%)
Myocardial infarction (yes/no)	10/146 (6.4%/93.6%)
Heart failure (yes/no)	64/92 (41%/59%)
Peripheral vascular disease (yes/no)	22/134 (14.1%/85.9%)
Cerebrovascular disease (yes/no)	12/144 (7.7%/92.3%)
Dementia (yes/no)	10/ 146 (6.4%/93.6%)
Connective tissue disease (yes/no)	5/151 (3.2%/96.8%)
Chronic obstructive pulmonary disease (yes/no)	25/131 (16%/84%)
Peptic ulcer disease (yes/no)	29/127 (18.6%/81.4%)
Liver disease (yes/no)	5/151 (3.2%/96.8%)
Hemiplegia (yes/no)	14/142 (9%/91%)
Solid tumor (yes/no)	18/138 (11.5%/88.5%)
Chronic renal insufficiency (yes/no)	38/118 (24.4%/75.6%)
Leukemia (yes/no)	6/150 (3.8%/96.2%)
Lymphoma (yes/no)	2/154 (1.3%/98.7%)

Table 2. Administered antibiotics (n = 156)

Antibiotic	Frequency (percentage)
Meropenem (aayes/no)	71/85 (45.5%/54.5%)
Imipenem/cilastatin (yes/no)	22/134 (14.1%/85.9%)
Piperacillin/tazobactam (yes/no)	12/144 (7.7%/92.3%)
Vancomycin (yes/no)	82/74 (52.6%/47.4%)
Teicoplanin (yes/no)	17/139 (10.9%/89.1%)
Linezolid (yes/no)	9/147 (5.8%/94.2%)
Colistimethate sodium (yes/no)	26/130 (16.7%/83.3%)
Tigecycline (yes/no)	20/136 (12.8%/87.2%)

Table 3. Mean laboratory values (n = 156)

Laboratory parameter	Mean ± SD, median, IQR	Units
Platelets	277.69 ±161, 263.5, 212	**×10⁹/L**
Urea	10.6 ± 10.8, 7.55, 7	**mmol/L**
Serum creatinine	145.71 ± 173.68, 79, 80	**µmol/L**
Leukocytes	13.78 ± 17.99, 11.2, 8.3	**×10⁹/L**
Neutrophils	9.09 ± 7.72, 7.94, 6.2	**×10⁹/L**
ALT	65.68 ± 143.5, 22, 29	**U/L**
AST	76.68 ± 207.4, 27, 30	**U/L**
GGT	95.24 ± 121.2, 47.5, 98	**U/L**
Total bilirubin	25.9 ± 51.9, 13.45, 10	**µmol/L**
Direct bilirubin	12.84 ± 35.2, 4.1, 4.8	**µmol/L**
Amylase	73.15 ± 84.95, 56, 64	**U/L**
Lipase	103.13 ± 290, 8, 42	**U/L**
CRP	133.65 ± 92.83, 125, 140	**mg/L**

Abbreviations: CRP – C-reactive protein; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; SD – standard deviation; IQR – interquartile range.

Table 4. Other relevant characteristics of the study population (n = 156)

Parameter	Frequency (percentage) for categorical variables, Mean ± SD, median, IQR for continuous variables
Adverse drug reaction (yes/no)	52/104 (33.3%/66.7%)
Recovery (yes/no)	33/123 (21.2%/78.8%)
Death (yes/no)	47/109 (30.1%/69.9%)
Prolonged hospitalization (yes/no)	11/145 (7.1%/92.9%)
Allergy history (yes/no)	21/135 (13.5%/86.5%)
ICU stay (yes/no)	74/82 (47.4%/52.6%)
Age	64.12 ± 13.75, 65.5, 17
Duration of ADR	2.81 ± 5.52, 0, 4
Charlson comorbidity index	4.5 ± 2.36, 4, 3
Duration of antibiotic therapy	9.68 ± 5.83, 9, 6
Length of hospitalization	36.58 ± 36.59, 29, 27
Total consultations	17.27 ± 17.24, 11, 15
Pharmacologist consultations	4.93 ± 3.99, 4, 5

Abbreviations: SD – standard deviation; IQR – interquartile range. ICU- Intensive Care Unit

Table 5. Characteristics of study participants according to the outcome – occurrence of ADRs.

Parameter	Cases (n=52) Frequency (%), median, [IQR]	Controls (n=104) Frequency (%), median, [IQR]	p-value
Sex (M/F)	33/19 (63.5%/36.5%)	65/39 (62.5%/37.5%)	**0.907**
Acute myocardial infarction	4/48(7.7%/92.3%)	6/98(5.8%/94.2%)	**0.644**
Heart failure	15/37(28.8/71.2%)	49/55(47.1%/52.9%)	**0.029**
Peripheral vascular disease	8/44(15.4%/84.6%)	14/90(13.5%/86.5%)	**0.745**
Cerebrovascular stroke	2/50(3.8%/96.2%)	10/94(9.6%/90.4%)	**0.202**
Dementia	3/49(5.8%/94.2%)	7/97(6.7%/93.3%)	**0.817**
Connective tissue disease	1/51(1.9%/98.1%)	4/100(3.8%/96.2%)	**0.520**
Peptic ulcer disease	5/47(9.6%/90.4%)	24/80(23.1%/76.9%)	**0.042**
Chronic obstructive pulmonary disease	7/45(13.5%/86.5%)	18/86(17.3%/82.7%)	**0.537**
Liver disease	3/49(5.8%/94.2%)	2/102(1.9%/98.1%)	**0.199**
Diabetes mellitus	16/36(30.8%/69.2)	34/70(32.7%/67.3%)	**0.808**
Hemiplegia	5/47(9.6%/90.4%)	9/95(8.7%/91.3%)	**0.843**
Chronic renal insufficiency	18/34(34.6%/65.4)	20/84(19.2%/80.8%)	**0.035**
Solid tumor	5/47(9.6%/90.4%)	13/91(12.5%/87.5%)	**0.595**
Leukemia	0/52(0%/100%)	6/98(5.8%/94.2%)	**0.077**
Lymphoma	0/52(0%/100%)	2/102(1.9%/98.1%)	**0.314**
Allergy	4/48(7.7%/92.3%)	17/87(16.3%/83.7%)	**0.135**
Meropenem	23/29(44.2%/55.8)	48/56(46.2%/53.8%)	**0.820**
Imipenem	8/44(15.4%/84.6%)	14/90(13.5%/86.5%)	**0.745**
Piperacillin/tazobactam	5/47(9.6%/90.4%)	7/97(6.7%/93.3%)	**0.524**
Vancomycin	24/28(46.2%/53.8)	58/46(55.8%/44.2%)	**0.257**
Teicoplanin	3/49(5.8%/94.2%)	14/90(13.5%/86.5%)	**0.146**
Linezolid	2/50(3.8%/96.2%)	7/97(6.7%/93.3%)	**0.466**
Colistin	10/42(19.2%/80.8)	16/88(15.4%/84.6%)	**0.543**
Tigecycline	7/45(13.5%/86.5%)	13/91(12.5%/87.5%)	**0.866**
ICU stay	27/25(51.9%/48.1)	47/57(45.2%/54.8%)	**0.427**
Recovery	33/19(63.5%/36.5)	0/104(0%/100%)	**0.000**

Parameter	Cases (n=52) Frequency (%), median, [IQR]	Controls (n=104) Frequency (%), median, [IQR]	p-value
Death	15/37(28.8%/71.2)	32/72(30.8%/69.2%)	**0.805**
Prolonged hospitalization	11/41(21.2%/78.8)	0/104(0%/100%)	**0.000**
Age	64.5[16]	67.1[19]	**0.507**
Duration of ADR	7[8]	0[0]	**0.000**
Charlson comorbidity index	4[3]	4.5[3]	**0.436**
Duration of antibiotic therapy	6.5[8]	10[5]	**0.005**
Length of hospitalization	30[26]	28.5[26]	**0.801**
Total consultations	12[14]	11[16]	**0.509**
Pharmacologist consultations	4[6]	4[4]	**0.722**
AST	30[29]	26[31]	**0.719**
ALT	22[30]	21.5[30]	**0.328**
GGT	50.5[116]	40.5[97]	**0.594**
Total bilirubin	12[9.7]	13.9[11.1]	**0.293**
Direct bilirubin	3.55[4.1]	4.1[4.9]	**0.344**
Amylase	61.5[47]	49[62]	**0.250**
Urea	7.75[7.7]	7.4[7]	**0.870**
Creatinine	86.5[135.8]	77[65]	**0.307**
Leukocytes	9.5[6.5]	11.7[9.3]	**0.037**
Neutrophils	6.8[4.8]	8.52[6.64]	**0.068**
Platelets	256[166]	267[241]	**0.936**
CRP	133.3[149.5]	120.4[116.1]	**0.897**

Abbreviations: CRP – C-reactive protein; ALT – alanine aminotransferase; AST – aspartate aminotransferase; GGT – gamma-glutamyl transferase; ICU- Intensive Care Unit; IQR – interquartile range.

Table 6. Statistically significant predictors of ADR occurrence identified by multivariate logistic regression

Risk factor	Crude OR (95% CI)	Adjusted OR (95% CI)	P value
Heart failure	**0.455** (0.223-0.928)	**0.262** (0.115-0.599)	0.002
Peptic ulcer disease	**0.355** (0.127-0.992)	**0.268** (0.091-0.790)	0.017
Chronic renal insufficiency	**2.224** (1.049-4.713)	**3.356** (1.415-7.955)	0.006

Abbreviations: OR – odds ratio; CI – confidence interval; p – probability of the null hypothesis.

DISCUSSION

Our study evaluated factors associated with ADRs related to reserve antibiotics in hospitalized patients in a tertiary healthcare institution. Our findings indicate that chronic renal insufficiency represents a significant risk factor for the occurrence of ADRs, while heart failure and peptic ulcer disease were associated with a lower risk of these reactions.

Previous studies have found that comorbidities represent an important factor influencing the development of ADRs (20,21). In the present study, chronic renal insufficiency was identified as a significant risk factor for the occurrence of ADRs. This finding is consistent with previous research showing that impaired renal function may increase the risk of drug accumulation, toxicity, and altered pharmacokinetics of many antimicrobial agents. Consequently, careful dose adjustment and monitoring of renal function are essential when prescribing reserve antibiotics to patients with renal impairment (22).

Reduced glomerular filtration, tubular secretion, and reabsorption in patients with chronic kidney disease lead to altered pharmacokinetics and increased systemic exposure to

drugs that are primarily eliminated through the kidneys. Accumulation of antibiotics and their metabolites may occur, increasing the likelihood of toxic effects and adverse reactions (23). Furthermore, uremia may alter protein binding of drugs due to decreased serum albumin levels, resulting in a higher fraction of pharmacologically active free drug in circulation (24). These mechanisms may partly explain the increased incidence of ADRs observed in patients with chronic renal insufficiency in our study.

On the other hand, heart failure and peptic ulcer disease were identified as protective factors in the multivariate analysis. One possible explanation for this finding is that patients with these conditions are more frequently monitored and receive more cautious pharmacotherapy due to their already recognized clinical vulnerability. Increased clinical attention and closer therapeutic monitoring may therefore contribute to a lower observed incidence of ADRs in these patients.

In our study, vancomycin and meropenem were the most frequently prescribed antibiotics. This observation reflects current clinical practice in tertiary care hospitals, where these agents are commonly used in the treatment of severe infections caused by multidrug-resistant microorganisms. However, the use of these antibiotics is often associated with a higher risk of adverse reactions due to their pharmacological properties, potential nephrotoxicity, and the complexity of patients' clinical conditions. A major concern is high dosage, since plasma trough levels of vancomycin show a linear association with the risk of acute kidney injury and is significantly correlated with nephrotoxicity, particularly at dosages of >20 mg/L or >4 g/day. Additionally, it has been discovered that a high dose of vancomycin (≥15 mg/L) is an independent risk factor for nephrotoxicity (25).

An important aspect of this study is the role of clinical pharmacologists in the detection, assessment, and prevention of ADRs. In our study, ADRs were identified through routine consultations and spontaneous reporting, highlighting the importance of integrating clinical pharmacology consultations into everyday hospital practice. Clinical pharmacologists contribute to early recognition of ADRs, optimization of pharmacotherapy, and individualized dose adjustment, particularly in high-risk patients receiving reserve antibiotics (26). The effectiveness of these interventions depends not only on the identification of drug-related problems but also on the successful implementation of recommendations in clinical practice. In line with previous studies, clinical pharmacologists and pharmacists play a crucial role in improving medication safety among hospitalized patients. Their recommendations are more likely to be accepted when they address clinically evident ADRs or involve high-risk drug combinations, particularly in the context of regularly prescribed therapies (27).

The findings of this study have important clinical implications. Identifying patients at increased risk for ADRs can help clinicians implement targeted monitoring strategies, adjust antibiotic dosing more appropriately, and potentially reduce the occurrence of adverse events. This is particularly relevant in tertiary healthcare settings where reserve antibiotics are frequently used in critically ill patients.

Limitations

Several limitations of this study should be acknowledged. First, the relatively small sample size may limit the generalizability of the findings. Second, the study was conducted in a single tertiary healthcare institution, which may reduce external validity. In addition, the retrospective component of the data collection may have introduced information bias due to incomplete or inconsistent medical records. Future studies with larger sample sizes and multicenter designs are needed to further clarify the risk factors associated with ADRs and to develop strategies for improving the safe use of reserve antibiotics in clinical practice.

CONCLUSION

The use of reserve antibiotics in hospitalized patients at tertiary healthcare institutions is associated with a measurable risk of ADRs, which is likely related to the severity of illness and complexity of these patients. Identification of risk factors may contribute to safer and more individualized antibiotic therapy in hospital settings.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. European Medicines Agency. Adverse drug reaction [Internet]. EMA; [cited 2026 Mar 5]. Available from: https://www.ema.europa.eu/en/glossary-terms/adverse-drug-reaction
2. Coleman JJ, Pontefract SK. Adverse drug reactions. Clin Med. 2016;16(5):481–5. doi:10.7861/clinmedicine.16-5-481
3. Kommu S, Carter C, Whitfield P. Adverse Drug Reactions. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 [cited 2026 Mar 5]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK599521/
4. Del Pozzo-Magaña BR, Liy-Wong C. Drugs and the skin: A concise review of cutaneous adverse drug reactions. Br J Clin Pharmacol. 2024;90(8):1838-1855. doi:10.1111/ bcp.15490.
5. Pavan G, Kumar M, Murti K, Dhingra S, Ravichandiran V. Exploring the factors influencing the health-related quality of life in patients experiencing adverse drug reactions: a cross-sectional study. J Patient Rep Outcomes. 2024;8(1):112. doi:10.1186/s41687-024-00790-0
6. Trivalle C, Burlaud A, Ducimetière P. Risk factors for adverse drug events in hospitalized elderly patients: A geriatric score. Eur Geriatr Med. 2011;2(5):284–9. doi:10.1016/j.eurger.2011.07.002

7. Alomar MJ. Factors affecting the development of adverse drug reactions (Review article). Saudi Pharm J SPJ. 2014;22(2):83–94. doi:10.1016/j.jsps.2013.02.003
8. Won SH, Suh SY, Yim E, Ahn HY. Risk Factors Related to Serious Adverse Drug Reactions Reported through Electronic Submission during Hospitalization in Elderly Patients. Korean J Fam Med. 2022;43(2):125–31. doi:10.4082/kjfm.21.0086
9. Harsch S, Tripathi S, Venuturumilli R, K SP, Reddy GHV, Mukherjee B, et al. Adverse Drug Reactions and Drug Interactions in Multimorbid Patients: A Review of Current Evidence. Cureus. 2025;17(11):e97640. doi: 10.7759/cureus.97640
10. Sharma L, Prakash A, Medhi B. Ensuring medication and patient safety for better quality healthcare. Indian J Pharmacol. 2024;56(6):375–8. doi:10.4103/ijp.ijp_109_25
11. Mohsen S, Dickinson JA, Somayaji R. Update on the adverse effects of antimicrobial therapies in community practice. Can Fam Physician. 2020;66(9):651–9.
12. Soraci L, Cherubini A, Paoletti L, Filippelli G, Luciani F, Laganà P, et al. Safety and Tolerability of Antimicrobial Agents in the Older Patient. Drugs Aging. 2023;40(6):499–526. doi:10.1007/s40266-023-01019-3
13. Jung IY, Kim JJ, Lee SJ, Kim J, Seong H, Jeong W, et al. Antibiotic-Related Adverse Drug Reactions at a Tertiary Care Hospital in South Korea. BioMed Res Int. 2017;2017:4304973. doi:10.1155/2017/4304973
14. Ledger EVK, Sabnis A, Edwards AM. Polymyxin and lipopeptide antibiotics: membrane-targeting drugs of last resort. Microbiology. 2022;168(2):001136. doi:10.1099/mic.0.001136
15. Gajic I, Tomic N, Lukovic B, Jovicevic M, Kekic D, Petrovic M, et al. A Comprehensive Overview of Antibacterial Agents for Combating Multidrug-Resistant Bacteria: The Current Landscape, Development, Future Opportunities, and Challenges. Antibiotics. 2025;14(3):221.doi:10.3390/antibiotics14030221
16. Patra M, Gupta AK, Kumar D, Kumar B. Antimicrobial Resistance: A Rising Global Threat to Public Health. Infect Drug Resist. 2025;18:5419–37. doi:10.2147/IDR.S530557
17. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis. 1987;40(5):373-83. doi: 10.1016/0021-9681(87)90171-8
18. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41(4):1149–60. doi:10.3758/BRM.41.4.1149
19. Lee EY, Gomes T, Drucker AM, Daneman N, Asaf A, Wu F, et al. Oral Antibiotics and Risk of Serious Cutaneous Adverse Drug Reactions. JAMA. 2024;332(9):730–7. doi:10.1001/jama.2024.11437
20. Bassi PU, Osakwe AI, Ogar CK, Elagbaje C, Nwankwo BB, Balogun ST, et al. Impact of comorbidity on adverse drug reaction profile in a cohort of patients treated with Artemisinin combination therapies for uncomplicated malaria in Nigeria. Pharmacol Res Perspect. 2017;5(2):e00302. doi:10.1002/prp2.302
21. Petrović NZ, Pejčić AV, Milosavljević MN, Janković SM. Risk factors for severe adverse drug reactions in hospitalized patients. Open Med. 2025;20(1):20241122. doi:10.1515/med-2024-1122
22. Kyriakopoulos C, Gupta V. Renal Failure Drug Dose Adjustments. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2026 [cited 2026 Mar 9]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK560512/
23. Zoccali C, Mallamaci F, De Caterina R. Pharmacokinetic relevance of glomerular hyperfiltration for drug dosing. Clin Kidney J. 2023;16(10):1580-1586. doi:10.1093/ckj/sfad079.
24. Liabeuf S, Berdougo-Tritz J, Augey L, Mbarek A, Jadoul M, Deray G, et al. Drug Exposure in Chronic Kidney Disease: It Is Not Just About the Glomerular Filtration Rate. Fundam Clin Pharmacol. 2025;39(4):e70037. doi:10.1111/fcp.70037
25. Alhassani RY, Bagadood RM, Balubaid RN, Barno HI, Alahmadi MO, Ayoub NA. Drug Therapies Affecting Renal Function: An Overview. Cureus. 2021;13(11):e19924. doi:10.7759/cureus.19924
26. Williams D. Monitoring medicines use: the role of the clinical pharmacologist. Br J Clin Pharmacol. 2012;74(4):685–90. doi:10.1111/j.1365-2125.2012.04316.x
27. Taegtmeyer AB, Curkovic I, Corti N, Rosen C, Egbring M, Russmann S, et al. Drug-related problems and factors influencing acceptance of clinical pharmacologists' alerts in a large cohort of neurology inpatients. Swiss Med Wkly. 2012;142(2728):w13615–w13615. doi:10.4414/smw.2012.13615

HOST DEFENSE PEPTIDES IN BRIEF

Suzana Popovic

University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Microbiology and Immunology

Received: 10.10.2021.
Accepted: 12.12.2021.

Corresponding author:

Suzana Popovic

Faculty of Medical Sciences, University of Kragujevac, Department of Microbiology and Immunology, Svetozara Markovica 69, 34000 Kragujevac, Serbia

E-mail: milosavljevicmilos91@gmail.com

ABSTRACT

Host defense peptides (HDPs) are an indispensable part of innate immunity. These multifunctional peptides, immanent in all living organisms, have diverse roles in host defense due to their antimicrobial and immunomodulatory properties. HDPs show activity against a broad spectrum of Gram+ and Gram- bacteria, fungi, mycobacteria, and some enveloped viruses. Their numerous roles as effector molecules of innate immune response and a link between innate and adaptive immunity, contribute to their antimicrobial properties. Furthermore, HDPs conduce to the resolution of inflammation and participate in wound healing. The increasing incidence of antimicrobial drug resistance and the need for agents that can modulate immune response have put these peptides into the spotlight of researchers' interest as potential therapeutics. In this review, we will present principal informations about the properties and the functions of host defense peptides, as well as their prospective applications.

Keywords: *Host defense peptides; antimicrobial; antitumor; immunomodulatory.*

UDK:

Eabr 2026; 27(2):187-197
DOI:10.2478/ sjecr-2022-0088

INTRODUCTION

Host defense peptides (HDPs), found in almost all species, from bacteria to humans, represent a heterogeneous group of peptides with a multiplicity of properties. Although they vary in amino acid sequences and structure, some features are in common for all members of this family. They are composed of 12 to 50 amino acids with a positive net charge (+ 2 to + 9) due to lysine or arginine residues, and about 50% hydrophobic residues. HDPs have diverse biological and functional properties, still, the most common characteristic is their antimicrobial effect. Cationic and amphiphilic features enable strong interaction with membranes, especially with negatively charged membranes of microorganisms. Host defense peptides with antimicrobial properties are also referred to as antimicrobial peptides (AMPs). HDPs are most often bifunctional. In addition to their broad-spectrum antimicrobial action, HDPs exert diverse immunomodulatory activities, both pro-inflammatory and anti-inflammatory. As such, HDPs are the first-line defense against pathogens and an indispensable part of innate immunity.

Physiologically active cationic peptides were first described in 1956 by Skarnes and Hirsch as peptides with antimicrobial activity (1, 2). Ten years later Zeya and Spitznagel introduced term defensins for several basic proteins found in polymorphonuclear leukocyte lysosomes, due to their participation in host defense (3, 4). At the beginning of the 1980's, in studies of the African frog Xenopus laevis oocyte system, it was observed that despite the nonsterile surgical procedures, cut margins of the wound didn't develop an infection, and normal healing of lesions occurred. Later on, Zasloff extracted two components with antibacterial activity from the skin of Xenopus laevis and called them "magainins" (5). Since then, a large number of natural peptides displaying a diversity of functions related to host defense have been isolated from cells and tissues of virtually all living organisms. They were found to be present in bacteria, fungi, and in all multicellular organisms as an inherent part of non-specific immunity. In bacteria, these molecules, called bacteriocins, provide survival in nutrient-poor environments by inhibiting adjacent microorganisms (6). Herbal peptides with the activities toward phytopathogens have been found in all plant organs. In mammals peptides participating in host defense reside in granulocytes, skin, and mucous membranes of the gastrointestinal, urogenital and respiratory tract. The antimicrobial peptide database (APD, http://aps.unmc.edu/AP/main.php) so far contains 337 bacteriocins, 18 fungi, 346 plant, and 2264 animal host defense peptides with antimicrobial and immunomodulatory activities.

CLASSIFICATION

HDPs are classified based on their primary and secondary structure. In mammals, three major families have been described: defensins, cathelicidins, and histatins (7). Defensins are further divided into three groups: α-defensins, β-defensins, and θ-defensins. They are widely expressed on the skin and mucous membranes, in Paneth's cells of the small intestine and stored in granules of neutrophils. The cathelicidins, found only in mammals, are immanent mostly in lymphoid tissue, but are also found in epithelial and brain tissue. The only cathelicidin found in humans is LL-37 (8). This multifunctional peptide is present predominantly in leukocytes, but also in different cells of various tissues and body fluids. Histatins are histidine-rich peptides present in saliva and, due to their bactericidal and fungicidal activity, participate in maintaining of oral health.

ANTIMICROBIAL ACTIVITY

The first contact between the peptide and bacteria is electrostatic. Peptidoglycan and cell wall-associated proteins, as well as cytoplasmic membrane surface, bear negative net charges. Electrostatic attraction between the negatively charged bacterial envelope and cationic peptides leads to peptide accumulation in the cell wall and interaction with the membrane surface. After binding to the membrane some peptides disrupt its structure and thus increase membrane permeability, or form pores, which leads to cell lysis. Others traverse the membrane, enter the cell and bind to vital molecules, inhibit the activity of enzymes participating in the synthesis of the cell wall, DNA, RNA, and proteins (9-11), or activate microbial autolytic systems (12). Since the surface of bacteria is more negatively charged than the eukaryotic cell membrane, peptides show a selective affinity for bacteria. Cholesterol present in the membrane of eukaryotic cells contributes to HDPs selectivity as well, by preventing peptide binding.

There are several mechanisms of HDPs antiviral action. They can act directly on the viral envelope (13) or interfere with viral adsorption and/or entry into the cell (14). Some HDPs that have the ability to translocate through the cytoplasmic membrane of eukaryotic cells, or others, that are produced and stored in cellular organelles, can activate host antiviral mechanisms inside an infected cell, or block expression of viral genes (15, 16). α-defensin HNP-1 inhibits replication of HIV-1 and influenza virus and inactivates vesicular stomatitis virus, adenovirus, HSV, CMV, and papillomavirus (17-19). Some other HDPs, such as cathelicidin LL-37 and neutrophile peptide Alpha-defensin-1, block HIV-1 infection either through down-regulation of HIV-1 coreceptor CXCR4(20) or by inhibition of virus replication in infected cells (21). θ-defensin Retrocyclin 2 inhibits avian influenza H5N1 virus through interference with viral mRNA transcription (22).

A number of HDPs have shown strong fungicidal properties(23). Most HDPs, that affects a broad spectrum of microorganisms, including fungi, bacteria, and viruses, act nonspecifically and exert their activity by inducing membrane lysis (24-27). HDPs that have primarily or exclusively antifungal properties are less immanent. These peptides have diverse specific targets. Members of the echinocandin family are inhibitors of β-glucan synthase, the enzyme involved in the generation of the cell wall in *Candida*, *Aspergillus, Cryptococcus*, and *Pneumocystis* species. Preclinical and clinical

studies have shown these peptides and their analogs as promising agents for the treatment of invasive and systemic *Candida* and *Aspergillus* infections(28, 29). Synthase inhibitors, such as Nikkomycins, interrupt the synthesis of chitin, a cell wall component essential for the structural integrity of the fungus. These HDPs have shown significant activity against *Candida albicans, Candida immitis*, and *Blastomyces dermatides*(30, 31). Aureobasidins alter the assembly of actin and chitin and interrupt the synthesis of sphingolipids in *Candida* spp. and *Cryptococcus neoformans* (32, 33). Some members of the defensin family with marked antifungal effects target the fungus-specific membrane glucosylceramide and induce the generation of toxic reactive oxygen species (34, 35). Histatins exert strong candidacidal activity (36-38) and significant activity against *Cryptococcus neoformans* and *Aspergillus fumigates* (39, 40). Histatin 5 is highly selective for fungi, acting on specific intracellular targets (41).

Only a minority of HDPs have been tested against parasites known to cause infections in humans. Since the cell membrane of protozoan parasites has a higher proportion of anionic phospholipids than mammalian cells, it seems that the antiprotozoan action of HDPs is also based on electrostatic binding and disruption of the plasma membrane. NK-lysin, a peptide found in cytoplasmic granules of porcine cytotoxic T cells and natural killer cells (the human analog is termed granulysin), interacts with the membrane of erythrocytes infected with *Plasmodium falciparum*, permeabilizes it, and kills intracellular parasites (42). NK-lysin also potently inactivates *Trypanosoma cruzi* by permeabilization of the parasite plasma membrane (43). Magainins and cecropins tested against a variety of *Plasmodium* species (44) have the potential to inhibit the normal development of oocysts and disrupt the sporogonic development of *Plasmodium*. Bovine lactoferrin peptides exhibited potent *in vitro* antigiardial activity (45).

The mechanisms of HDPs antimicrobial activity are shown in Table 1.

Table 1. Mechanisms of HDPs antimicrobial activity

Antibacterial			
Mechanism of action	HDPs	Target organism	Reference
Permeabilization of the outer and the inner membrane	HNP4	*E. coli, E. aerogenes, En. faecalis, S. aureus, B. cereus*	(46)
Membrane dysfunction	Lactoferrin	*V. cholerae, S. typhimurium, E. coli.*	(47)
Inhibition of cell wall synthesis	BMAP-28	*A. baumannii*	(48)
	Polymixin E	*E. coli, P. aeruginosa*	(49)
	RTD-1	*E. coli, P. aeruginosa, S. aureus*	(50)
	Mersacidin	*B. cereus M. luteus S. simulans*	(51) (52)
	Seminalplasmin	*E. coli*	(53)
	HNP-1	*C. difficile*	(54)
	hBD3	*S.aureus, E. faecium*	(55) (10)
Inhibition of DNA, RNA and protein synthesis	tPMP-1 and tPMP-2	*S.aureus*	(11)
	Pleurocidin	*E. coli*	(56)
	Apidaecins, oncocins	*E. coli*	
Activation of the microbial autolytic systems	RTD-1 and RTD-2	*S. aureus, S. simulans, S. carnosus*	
Antiviral			
Viral envelopes lysis	LL-37	*Vaccinia virus*	(13)
Interference with viral adsorption and/or entry into the cell	Dermaseptins	HSV1	(14)
	HNPs-1 - 3, HD-5	HPV	(17)
	HNP-1	HSV1	(18)
	NP-1	HSV2	(19)
	HD5	HIV1	(57)
	β-defensin-2 and HBD-3	HIV1	(58)
Inhibition of replication	Melittin, Cecropin	HIV1	(15)
	Alpha-defensin-1	HIV1	(20)
	β-defensin-2, HBD-3	HIV1	(58) (21)
	LL-37	HIV1	(59)
Blocking expression of viral genes	HNP-1 and HBD-2	HIV1	(16)
	LL-37, mCRAMP	*Influenza A virus*	(22)
	Retrocyclin 2	H5N1	(60)
	HNP2, HDP	HSV2	

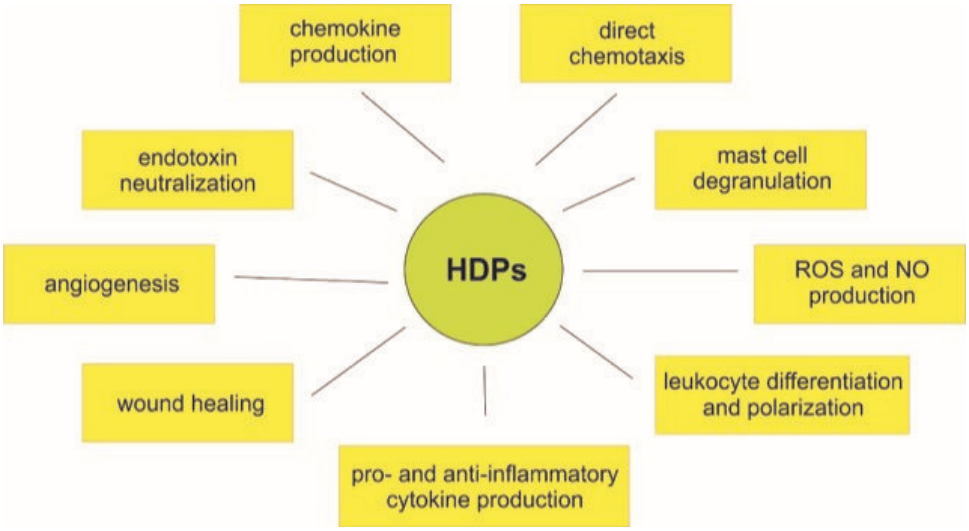
Antifungal			
Membrane lysis	Cecropin B, Dermaseptin	*A. flavus, A. fumigates, A. niger, F. moniliforme, F. oxysporum*	(24)
	Ranalexin, Magainin II	*Candida spp, Cryptococcus*	(27)
Inhibition of fungal chitin synthase	Nikkomycins X and Z	*C. immitis, B. dermatitidis*	(30, 31)
Inhibition of sphingolipid synthesis	Aureobasidin A	*S. cerevisiae*	(32)
Disruption of actin assembly and chitin delocalization	Aureobasidin A	*S. cerevisiae*	(61)
Induction of reactive oxygen species	RsAFP2	*C. albicans*	(62) (63)
	HsAFP1	*S. cerevisiae*	(64)
Induction of ion fluxes	DmAMP1		(65)
Cell cycle progression impairment	RsAFP2	*N. crassa*	(66)
	Psd1	*N. crassa*	
Antiparasitic			
Membrane permeabilisation	NK-lysin	*P. falciparum*	(42)
		T. cruzi	(43)
Oocyst development and sporozoite production	Magainin 2, Cecropin B	*Plasmodium spp.*	(44)
Membrane disruption, morphological changes, apoptosis induction	bLF	*G. intestinalis*	(45)

ROLE IN IMMUNITY

In addition to antimicrobial potency, HDPs have a variety of functions related to host defense (Figure 1.). They act as signaling molecules that coordinate innate and adaptive immune response and promote subsequent healing of injured tissue and neovascularization of the wound (67-71). Their numerous immunomodulatory functions include the ability to act as chemokines and/or to induce the production of chemokines that attract neutrophils, monocytes, T cells, and mast cells to the infection site (72). HDPs stimulate mast cell degranulation which results in increased permeability of blood vessels and enables the recruitment of leukocytes to the site of inflammation (73, 74). In an inflammatory environment, they increase the production of ROS and amplify the respiratory burst in macrophages (75) and neutrophils (76). HDPs can modulate host gene expression (77) thus influencing the production of pro- and anti-inflammatory cytokines. Also, they may facilitate maturation and function of dendritic cells and enhance adaptive immune response (78). Importantly, these peptides can bind LPS and neutralize them, or block the binding of LPS to macrophage receptors (79, 80), thus preventing an excessive proinflammatory response that can result in tissue damage (81).

The immunomodulatory activity of HDPs is not unidirectional. Depending on stimuli, HDPs can exert a dual impact on the immune response. Popovic et al. have shown that brevinin-2-related peptide-ERa (B2RPERa), isolated from *Hylarana erythraea*, induces proliferation of resting peripheral blood mononuclear cells, but limits proliferative response to concanavalin A (82). The dual effect of LL-37 is well documented. *In vitro* experiments showed that this cathelicidin, expressed in human myeloid and epithelial cells, completely inhibited the expression of proinflammatory genes, but did not affect the expression of chemotactic mediators in LPS-activated macrophages (83). Furthermore, this peptide influenced the differentiation of dendritic cells: LL-37 increased their endocytic capacity, modified expression and function of receptors involved in phagocytosis, increased expression of costimulatory molecules and production of Th1 cytokines, promoting Th1 cell response (84). In contrast, simultaneous treatment of immature dendritic cells with LL-37 and TLR-ligands inhibited their maturation (85). As studies have shown that the expression of this peptide is increased during infection or inflammation, it seems that LL-37, and possibly some other HDPs, acts as a negative feedback signal that promotes local immune response to infection, and prevents systemic hyperproliferative response. The crucial role of HDPs in both prevention and resolution of infection has also been shown in animal models (81, 86, 87).

Figure 1. Immunomodulatory properties



ANTITUMOR ACTIVITY

In recent years it became evident that HDPs exhibit promising antitumor activity and may have therapeutic value. A variety of peptides tested *in vitro* for their ability to kill cancer cells showed an outstanding direct cytotoxic effect on leukemia, lymphoma, bladder, ovarian, cervical, breast, lung, and oral squamous carcinoma cells (88-96). The advantage of HDPs over conventional chemotherapeutics is their selectivity for cancer cells. That discrimination is based on the difference in expression of cell surface molecules such as phosphatidylserine, heparan sulfate, or sialic acid (97, 98), variance in membrane fluidity, and the presence of a high number of microvilli that increase the surface of the cancer cell. Interaction between HDPs and cancer cells is electrostatic. Owing to their cationic amphipathic structure, HDPs interact with negatively charged molecules that are more abundantly expressed on cancer cells. Damaging of the cell membrane can be apparent within minutes. Certain HDPs do not disturb cell membrane integrity, but translocate through the membrane of the cancer cell to the cytosol and interact with cellular components, preferentially with mitochondria whose membrane contains more phosphatidylserine compared to a normal cell, and trigger apoptosis (99). Conducively, cancer cells change the microenvironment lowering pH, and that can be of benefit for optimal activity of HDPs. Since HDPs kill cancer cells rapidly in non-receptor mediated mode, targeting molecules that have important roles in the progression of cancer, the emergence of resistance is less possible. In addition to the direct cytotoxic effect, HDPs participate in the immune response to cancer as a part of innate immunity and the connection between innate and adaptive immune response (100).

Based on *in vitro* experiments, a variety of HDPs has been tested *in vivo* in animal models. A rising number of *in vivo* studies on mice showed that certain HDPs administered intratumorally (101, 102), peritumorally (103), subcutaneously (104), or applied in the form of sprays or ointment in case of melanoma (105) had direct antitumor activity, inhibited tumor growth and increased the survival time of experimental animals. Intratumoral injections of NRC-03 and NRC-07, a pleurocidin family HDPs, to breast cancer-bearing immune-deficient NOD SCID mice resulted in significant inhibition of tumor growth. Furthermore, histologic analysis of peptide-treated tumors revealed larger necrotic zones than that of control animals. Importantly, intratumoral delivery of peptides had no deleterious side-effects (106). Shan et al. have shown that five days of administration of magainin II-bombesin conjugate directly in the tumor of mice bearing MCF-7 tumor grafts resulted in reduced tumor masses alongside extensive necrotic areas (107).

Since therapeutic use of HDPs requires maintaining of *in situ* concentrations sufficient to kill cancer cells, new approaches to overcome this problem involve vector-mediated delivery of genes encoding HDPs into cancer cells (108), or targeting cancer cells with immunoconjugates containing HDPs (109). Importantly, the membranolytic activity of HDPs and the resulting increase in permeability of cancer cell membrane can be used to enhance the cytotoxic activity of conventional chemotherapeutics (110, 111).

TROUBLESHOOTING

The role of HDPs in host defense is complex and their multiple functions can be utilized for designing future therapeutic agents. However, there are several crucial limitations to their therapeutic use. One of the problems is the insufficient specificity of certain HDPs. While some HDPs selectively kill microorganisms and cancer cells, others are more or less cytotoxic for healthy eucaryotic cells and may have severe hemolytic activity. Consequently, administration of HDPs may result in systemic toxicity.

One of the prerequisites for the therapeutic use of HDPs is sufficient stability that will allow peptides to complete

their action. Fast inactivation by gastrointestinal enzymes rule out oral administration. In the blood, HDPs are bind and inactivated by serum albumins and low-density proteins, or degraded by serum enzymes. The strategy for overcoming this problem is employment of different delivery systems like liposomes, nanoparticles, nanotubes, etc (112).

In addition, although HDPs exert their antimicrobial action in non-receptor mediated mode (113), several mechanisms leading to diminution of susceptibility to HDPs have been described in some fungi (114) and bacteria (115). Those mechanisms include reduction of negative net charge of outer envelopes, up-regulation of proteolytic enzymes that degrade or modify HDPs, or engagement of efflux pumps. The same strategies are found to be exploited by cancer cells as well.

One more obstacle that has to be overcome is the high cost of peptide production. Additionally, chemical synthesis has to provide high yield and high purity of correctly folded peptides. Nowadays recombinant technology, using bacteria, yeast, plant, and mammalian cells as host cells, is employed in most for cost-effective production of large-scale peptides.

Currently, a number of investigations are directed toward designing peptide analogs with advanced performances. As the features and the functions of HDPs are determined by amino acid composition, modifications of peptide structure have been utilized to enhance desired functions and to modulate other properties, such as selectivity and stability (116-120). Excision, insertion, or substitution of amino acid residues in native peptides can effectively improve electrostatic attraction and consequently selectivity for microorganisms or cancer cells, along with stability and resistance to proteases. Another possibility that is intensively investigated is the creation of truncated analogs attained by the excision of biologically active regions of natural HDPs. In recent years combinatorial libraries are employed for computer-aided modeling and designing small peptides with better performances relative to naturally occurring HDPs (121, 122). A number of new HDP analogs with enhanced properties have been generated and tested in ongoing laboratory studies (123-125) and clinical trials. Omiganan, an indolicidin analog with antimicrobial and anti-inflammatory activities, is in phase III clinical trials for the prevention of catheter infections (126) and phase II trials for the treatment of papillomavirus-induced genital lesions (127). Pexiganan (a synthetic variant of magainin) is currently tested in phase III clinical studies for the treatment of infections of diabetic foot ulcers (128). PAC-113, derived from histatin found in human saliva, is investigated for the treatment of oral candidiasis in immunocompromised patients (129). hLF1-11, derived from human lactoferrin, along with antimicrobial activity, has demonstrated immunomodulatory properties (130), and currently, its efficacy in the protection and prevention of infection during allogeneic stem cell transplantation is assessing in clinical trials. Oncolytic peptide LTX-315, derived from the segment of bovine lactoferricin that has been identified as important for its anticancer properties, in phase I/II studies showed strong and selective anticancer activity (131, 132).

CONCLUSION

The emergence of antimicrobial drug resistance, lack of adequate treatment for some infectious diseases, insufficient efficiency of anticancer drugs, the increasing emergence of the diseases that originate from disturbed immunity, are unsolved problems of modern medicine. The abundance of HDPs in living organisms and the multiplicity of their functions in host defense offer the paramount possibilities. Despite the aforementioned problems, these peptides can be used at least as the models for designing improved, more efficient molecules, with necessary therapeutic properties.

ACKNOWLEDGMENTS

This study was supported by the Faculty of Medical Sciences, University of Kragujevac (project JP 08/11).

CONFLICT OF INTEREST

The author have no conflicts of interest to declare.

REFERENCES

1. Skarnes RC, Watson DWJBr. Antimicrobial factors of normal tissues and fluids. Bacteriol Rev 1957;21(4):273.
2. Hirsch JGJTJoem. Phagocytin: a bactericidal substance from polymorphonuclear leucocytes. J Expt Med 1956;103(5):589.
3. Zeya H, Spitznagel JK. Cationic proteins of polymorphonuclear leukocyte lysosomes I. resolution of antibacterial and enzymatic activities. J Bacteriol 1966;91(2):750-4.
4. Zeya H, Spitznagel JK. Cationic proteins of polymorphonuclear leukocyte lysosomes II. Composition, properties, and mechanism of antibacterial action. J Bacteriol 1966;91(2):755-62.
5. Zasloff M. Magainins, a class of antimicrobial peptides from Xenopus skin: Isolation, characterization of two active forms, and partial cDNA sequence of a precursor. Proc Natl Acad Sci USA 1988;23(2-3):360.
6. Riley MA, Gordon DM. The ecological role of bacteriocins in bacterial competition. Trends Microbiol 1999;7(3):129-33.
7. Brandenburg L-O, Merres J, Albrecht L-J, Varoga D, Pufe T. Antimicrobial peptides: multifunctional drugs for different applications. Polymers 2012;4(1):539-60.
8. De Smet K, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. Biotechnol Lett 2005;27(18):1337-47.
9. Yeaman MR, Yount NY. Mechanisms of antimicrobial peptide action and resistance. Pharmacol Rev 2003;55(1):27-55.
10. Patrzykat A, Friedrich CL, Zhang L, Mendoza V, Hancock RE. Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in Escherichia coli. Antimicrob Agents Chemother 2002;46(3):605-14.
11. Krizsan A, Volke D, Weinert S, Sträter N, Knappe D, Hoffmann R. Insect-derived proline-rich antimicrobial

peptides kill bacteria by inhibiting bacterial protein translation at the 70 S ribosome. Angew Chem Int Ed Engl 2014;53(45):12236-9.
12. Ginsburg I. Bactericidal cationic peptides can also function as bacteriolysis-inducing agents mimicking beta-lactam antibiotics?; it is enigmatic why this concept is consistently disregarded. Med Hypotheses 2004;62(3):367-74.
13. Aboudy Y, Mendelson E, Shalit I, Bessalle R, Fridkin M. Activity of two synthetic amphiphilic peptides and magainin-2 against herpes simplex virus types 1 and 2. Int J Pept Protein Res 1994;43(6):573-82.
14. Belaid A, Aouni M, Khelifa R, Trabelsi A, Jemmali M, Hani K. In vitro antiviral activity of dermaseptins against herpes simplex virus type 1. J Med Virol 2002;66(2):229-34.
15. Wachinger M, Kleinschmidt A, Winder D, von Pechmann N, Ludvigsen A, Neumann M, et al. Antimicrobial peptides melittin and cecropin inhibit replication of human immunodeficiency virus 1 by suppressing viral gene expression. J Gen Virol 1998;79 (Pt 4):731-40.
16. Barlow PG, Svoboda P, Mackellar A, Nash AA, York IA, Pohl J, et al. Antiviral Activity and Increased Host Defense against Influenza Infection Elicited by the Human Cathelicidin LL-37. PLOS ONE 2011;6(10):e25333.
17. Buck CB, Day PM, Thompson CD, Lubkowski J, Lu W, Lowy DR, et al. Human alpha-defensins block papillomavirus infection. Proc Natl Acad Sci U S A 2006;103(5):1516-21.
18. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. J Virol 1986;60(3):1068-74.
19. Sinha S, Cheshenko N, Lehrer RI, Herold BC. NP-1, a rabbit alpha-defensin, prevents the entry and intercellular spread of herpes simplex virus type 2. Antimicrob Agents Chemother 2003;47(2):494-500.
20. Chang TL, Vargas J, Jr., DelPortillo A, Klotman ME. Dual role of alpha-defensin-1 in anti-HIV-1 innate immunity. J Clin Invest 2005;115(3):765-73.
21. Bergman P, Walter-Jallow L, Broliden K, Agerberth B, Söderlund J. The antimicrobial peptide LL-37 inhibits HIV-1 replication. Curr HIV Res 2007;5(4):410-5.
22. Liang QL, Zhou K, He HX. Retrocyclin 2: a new therapy against avian influenza H5N1 virus in vivo and vitro. Biotechnol Lett 2010;32(3):387-92.
23. Durnaś B, Wnorowska U, Pogoda K, Deptuła P, Wątek M, Piktel E, et al. Candidacidal Activity of Selected Ceragenins and Human Cathelicidin LL-37 in Experimental Settings Mimicking Infection Sites. PLOS ONE 2016;11(6):e0157242.
24. De Lucca AJ, Bland JM, Jacks TJ, Grimm C, Walsh TJ. Fungicidal and binding properties of the natural peptides cecropin B and dermaseptin. Medical Mycology 1998;36(5):291-8.
25. Mangoni ML, Grovale N, Giorgi A, Mignogna G, Simmaco M, Barra D. Structure-function relationships in bombinins H, antimicrobial peptides from Bombina skin secretions. Peptides 2000;21(11):1673-9.
26. Mangoni ML, Marcellini HG, Simmaco M. Biological characterization and modes of action of temporins and bombinins H, multiple forms of short and mildly cationic anti-microbial peptides from amphibian skin. J Pept Sci 2007;13(9):603-13.
27. Giacometti A, Cirioni O, Barchiesi F, Del Prete MS, Scalise G. Antimicrobial activity of polycationic peptides. Peptides 1999;20(11):1265-73.
28. Schmatz DM, Romancheck MA, Pittarelli LA, Schwartz RE, Fromtling RA, Nollstadt KH, et al. Treatment of Pneumocystis carinii pneumonia with 1,3-beta-glucan synthesis inhibitors. Proceedings of the National Academy of Sciences of the United States of America 1990;87(15):5950-4.
29. Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. J Med Vet Mycol 1997;35(2):79-86.
30. Hector RF, Zimmer BL, Pappagianis D. Evaluation of nikkomycins X and Z in murine models of coccidioidomycosis, histoplasmosis, and blastomycosis. Antimicrob Agents Chemother 1990;34(4):587-93.
31. Clemons KV, Stevens DA. Efficacy of nikkomycin Z against experimental pulmonary blastomycosis. Antimicrob Agents Chemother 1997;41(9):2026-8.
32. Nagiec MM, Nagiec EE, Baltisberger JA, Wells GB, Lester RL, Dickson RC. Sphingolipid synthesis as a target for antifungal drugs. Complementation of the inositol phosphorylceramide synthase defect in a mutant strain of Saccharomyces cerevisiae by the AUR1 gene. J Biol Chem 1997;272(15):9809-17.
33. Takesako K, Kuroda H, Inoue T, Haruna F, Yoshikawa Y, Kato I, et al. Biological properties of aureobasidin A, a cyclic depsipeptide antifungal antibiotic. J Antibiot (Tokyo) 1993;46(9):1414-20.
34. Fehlbaum P, Bulet P, Michaut L, Lagueux M, Broekaert WF, Hetru C, et al. Insect immunity. Septic injury of Drosophila induces the synthesis of a potent antifungal peptide with sequence homology to plant antifungal peptides. J Biol Chem 1994;269(52):33159-63.
35. Thevissen K, Kristensen HH, Thomma BP, Cammue BP, François IE. Therapeutic potential of antifungal plant and insect defensins. Drug Discov Today 2007;12(21-22):966-71.
36. Santarpia RP, 3rd, Pollock JJ, Renner RP, Gwinnett AJ. In vivo antifungal efficacy of salivary histidine-rich polypeptides: preliminary findings in a denture stomatitis model system. J Prosthet Dent 1991;66(5):693-9.
37. Pollock JJ, Denepitiya L, MacKay BJ, Iacono VJ. Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on Candida albicans. Infect Immun 1984;44(3):702-7.
38. Rayhan R, Xu L, Santarpia RP, 3rd, Tylenda CA, Pollock JJ. Antifungal activities of salivary histidine-rich polypeptides against Candida albicans and other oral yeast isolates. Oral Microbiol Immunol 1992;7(1):51-2.
39. Helmerhorst EJ, Reijnders IM, van't Hof W, Simoons-Smit I, Veerman EC, Amerongen AV. Amphotericin B- and fluconazole-resistant Candida spp., Aspergillus fumigatus, and other newly emerging pathogenic fungi are

susceptible to basic antifungal peptides. Antimicrob Agents Chemother 1999;43(3):702-4.
40. Tsai H, Bobek LA. Human salivary histatin-5 exerts potent fungicidal activity against Cryptococcus neoformans. Biochim Biophys Acta 1997;1336(3):367-9.
41. Helmerhorst EJ, van't Hof W, Breeuwer P, Veerman EC, Abee T, Troxler RF, et al. Characterization of histatin 5 with respect to amphipathicity, hydrophobicity, and effects on cell and mitochondrial membrane integrity excludes a candidacidal mechanism of pore formation. J Biol Chem 2001;276(8):5643-9.
42. Gelhaus C, Jacobs T, Andrä J, Leippe M. The antimicrobial peptide NK-2, the core region of mammalian NK-lysin, kills intraerythrocytic Plasmodium falciparum. Antimicrob Agents Chemother 2008;52(5):1713-20.
43. Jacobs T, Bruhn H, Gaworski I, Fleischer B, Leippe M. NK-Lysin and Its Shortened Analog NK-2 Exhibit Potent Activities against Trypanosoma cruzi. Antimicrob Agents Chemother 2003;47:607-13.
44. Gwadz RW, Kaslow D, Lee JY, Maloy WL, Zasloff M, Miller LH. Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes. Infect Immun 1989;57(9):2628-33.
45. Aguilar-Diaz H, Canizalez-Roman A, Nepomuceno-Mejia T, Gallardo-Vera F, Hornelas-Orozco Y, Nazmi K, et al. Parasiticidal effect of synthetic bovine lactoferrin peptides on the enteric parasite Giardia intestinalis. Biochem Cell Biol 2017;95(1):82-90.
46. Ericksen B, Wu Z, Lu W, Lehrer RI. Antibacterial activity and specificity of the six human {alpha}-defensins. Antimicrob Agents Chemother 2005;49(1):269-75.
47. Ellison RT, 3rd, Giehl TJ. Killing of gram-negative bacteria by lactoferrin and lysozyme. J Clin Invest 1991;88(4):1080-91.
48. Guo Y, Xun M, Han J. A bovine myeloid antimicrobial peptide (BMAP-28) and its analogs kill pan-drug-resistant Acinetobacter baumannii by interacting with outer membrane protein A (OmpA). Medicine (Baltimore) 2018;97(42):e12832.
49. Poirel L, Jayol A, Nordmann P. Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes. Clin Microbiol Rev 2017;30(2):557-96.
50. Beringer PM, Bensman TJ, Ho H, Agnello M, Denovel N, Nguyen A, et al. Rhesus θ-defensin-1 (RTD-1) exhibits in vitro and in vivo activity against cystic fibrosis strains of Pseudomonas aeruginosa. J Antimicrob Chemother 2016;71(1):181-8.
51. Brötz H, Bierbaum G, Leopold K, Reynolds PE, Sahl HG. The lantibiotic mersacidin inhibits peptidoglycan synthesis by targeting lipid II. Antimicrob Agents Chemother 1998;42(1):154-60.
52. Chitnis SN, Prasad KS. Seminalplasmin, an antimicrobial protein from bovine seminal plasma, inhibits peptidoglycan synthesis in Escherichia coli. FEMS Microbiol Lett 1990;60(3):281-4.
53. Furci L, Baldan R, Bianchini V, Trovato A, Ossi C, Cichero P, et al. New role for human α-defensin 5 in the fight against hypervirulent Clostridium difficile strains. Infect Immun 2015;83(3):986-95.
54. Maisetta G, Batoni G, Esin S, Florio W, Bottai D, Favilli F, et al. In vitro bactericidal activity of human beta-defensin 3 against multidrug-resistant nosocomial strains. Antimicrob Agents Chemother 2006;50(2):806-9.
55. Xiong YQ, Bayer AS, Yeaman MR. Inhibition of intracellular macromolecular synthesis in Staphylococcus aureus by thrombin-induced platelet microbicidal proteins. J Infect Dis 2002;185(3):348-56.
56. Wilmes M, Stockem M, Bierbaum G, Schlag M, Götz F, Tran DQ, et al. Killing of staphylococci by θ-defensins involves membrane impairment and activation of autolytic enzymes. Antibiotics (Basel) 2014;3(4):617-31.
57. Furci L, Tolazzi M, Sironi F, Vassena L, Lusso P. Inhibition of HIV-1 infection by human α-defensin-5, a natural antimicrobial peptide expressed in the genital and intestinal mucosae. PLoS One 2012;7(9):e45208.
58. Quiñones-Mateu ME, Lederman MM, Feng Z, Chakraborty B, Weber J, Rangel HR, et al. Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. Aids 2003;17(16):F39-48.
59. Seidel A, Ye Y, de Armas LR, Soto M, Yarosh W, Marcsisin RA, et al. Cyclic and acyclic defensins inhibit human immunodeficiency virus type-1 replication by different mechanisms. PLoS One 2010;5(3):e9737.
60. Hazrati E, Galen B, Lu W, Wang W, Ouyang Y, Keller MJ, et al. Human alpha- and beta-defensins block multiple steps in herpes simplex virus infection. J Immunol 2006;177(12):8658-66.
61. Endo M, Takesako K, Kato I, Yamaguchi H. Fungicidal action of aureobasidin A, a cyclic depsipeptide antifungal antibiotic, against Saccharomyces cerevisiae. Antimicrob Agents Chemother 1997;41(3):672-6.
62. Aerts AM, François IE, Meert EM, Li QT, Cammue BP, Thevissen K. The antifungal activity of RsAFP2, a plant defensin from raphanus sativus, involves the induction of reactive oxygen species in Candida albicans. J Mol Microbiol Biotechnol 2007;13(4):243-7.
63. Aerts AM, Bammens L, Govaert G, Carmona-Gutierrez D, Madeo F, Cammue BP, et al. The Antifungal Plant Defensin HsAFP1 from Heuchera sanguinea Induces Apoptosis in Candida albicans. Front Microbiol. 2011;2:47.
64. Aerts AM, François IE, Bammens L, Cammue BP, Smets B, Winderickx J, et al. Level of M(IP)2C sphingolipid affects plant defensin sensitivity, oxidative stress resistance and chronological life-span in yeast. FEBS Lett 2006;580(7):1903-7.
65. Thevissen K, Ghazi A, De Samblanx GW, Brownlee C, Osborn RW, Broekaert WF. Fungal membrane responses induced by plant defensins and thionins. J Biol Chem 1996;271(25):15018-25.
66. Lobo DS, Pereira IB, Fragel-Madeira L, Medeiros LN, Cabral LM, Faria J, et al. Antifungal Pisum sativum defensin 1 interacts with Neurospora crassa cyclin F related to the cell cycle. Biochemistry 2007;46(4):987-96.
67. Hancock RE, Diamond G. The role of cationic antimicrobial peptides in innate host defences. Trends Microbiol 2000;8(9):402-10.

68. Beisswenger C, Bals R. Functions of antimicrobial peptides in host defense and immunity. Curr Protein Pept Sci 2005;6(3):255-64.
69. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol. 2004;75(1):39-48.
70. Sørensen OE, Cowland JB, Theilgaard-Mönch K, Liu L, Ganz T, Borregaard N. Wound healing and expression of antimicrobial peptides/polypeptides in human keratinocytes, a consequence of common growth factors. J Immunol 2003;170(11):5583-9.
71. Yang B, Suwanpradid J, Sanchez-Lagunes R, Choi HW, Hoang P, Wang D, et al. IL-27 Facilitates Skin Wound Healing through Induction of Epidermal Proliferation and Host Defense. J Invest Dermatol. 2017;137(5):1166-75.
72. Grigat J, Soruri A, Forssmann U, Riggert J, Zwirner J. Chemoattraction of Macrophages, T Lymphocytes, and Mast Cells Is Evolutionarily Conserved within the Human α-Defensin Family. J Immunol 2007;179(6):3958-65.
73. Toyoguchi T, Ebihara M, Ojima F, Hosoya J, Shoji T, Nakagawa Y. Histamine release induced by antimicrobial agents and effects of antimicrobial agents on vancomycin-induced histamine release from rat peritoneal mast cells. J Pharm Pharmacol 2000;52(3):327-31.
74. Befus AD, Mowat C, Gilchrist M, Hu J, Solomon S, Bateman A. Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action. J Immunol 1999;163(2):947-53.
75. Zughaier SM, Shafer WM, Stephens DS. Antimicrobial peptides and endotoxin inhibit cytokine and nitric oxide release but amplify respiratory burst response in human and murine macrophages. Cell Microbiol 2005;7(9):1251-62.
76. Ammar B, Périanin A, Mor A, Sarfati G, Tissot M, Nicolas P, et al. Dermaseptin, a peptide antibiotic, stimulates microbicidal activities of polymorphonuclear leukocytes. Biochem Biophys Res Commun 1998;247(3):870-5.
77. Xie H, Wei J, Qin Q. Antiviral function of Tachyplesin I against iridovirus and nodavirus. Fish Shellfish Immunol 2016;58:96-102.
78. Lillard J, Boyaka P, Chertov O, Oppenheim J, McGhee J. Mechanisms for induction of aquired host immunity by neutrophil peptide defensins. Proceedings of the National Academy of Sciences of the United States of America 1999;96:651-6.
79. Hancock RE, Scott MG. The role of antimicrobial peptides in animal defenses. Proc Natl Acad Sci U S A 2000;97(16):8856-61.
80. Giuliani A, Pirri G, Rinaldi AC. Antimicrobial peptides: the LPS connection. Methods Mol Biol 2010;618:137-54.
81. Scott MG, Davidson DJ, Gold MR, Bowdish D, Hancock RE. The human antimicrobial peptide LL-37 is a multifunctional modulator of innate immune responses. J Immunol 2002;169(7):3883-91.
82. Popovic S, Djurdjevic P, Zaric M, Mijailovic Z, Avramovic D, Baskic DJPb. Effects of host defense peptides B2RP, Brevinin-2GU, D-Lys-Temporin, Lys-XT-7 and D-Lys-Ascaphin-8 on peripheral blood mononuclear cells: Preliminary study. Periodicum Biologorum 2017;119(2).
83. Mookherjee N, Brown KL, Bowdish DM, Doria S, Falsafi R, Hokamp K, et al. Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. J Immunol 2006;176(4):2455-64.
84. Davidson DJ, Currie AJ, Reid GS, Bowdish DM, MacDonald KL, Ma RC, et al. The cationic antimicrobial peptide LL-37 modulates dendritic cell differentiation and dendritic cell-induced T cell polarization. J Immunol 2004;172(2):1146-56.
85. Kandler K, Shaykhiev R, Kleemann P, Klescz F, Lohoff M, Vogelmeier C, et al. The anti-microbial peptide LL-37 inhibits the activation of dendritic cells by TLR ligands. Int Immunol 2006;18(12):1729-36.
86. Brogden KA, Heidari M, Sacco RE, Palmquist D, Guthmiller JM, Johnson GK, et al. Defensin-induced adaptive immunity in mice and its potential in preventing periodontal disease. Oral Microbiol Immunol 2003;18(2):95-9.
87. Tani K, Murphy WJ, Chertov O, Salcedo R, Koh CY, Utsunomiya I, et al. Defensins act as potent adjuvants that promote cellular and humoral immune responses in mice to a lymphoma idiotype and carrier antigens. Int Immunol 2000;12(5):691-700.
88. Mader JS, Hoskin DW. Cationic antimicrobial peptides as novel cytotoxic agents for cancer treatment. Expert Opin Investig Drugs 2006;15(8):933-46.
89. Risso A, Zanetti M, Gennaro R. Cytotoxicity and apoptosis mediated by two peptides of innate immunity. Cell Immunol 1998;189(2):107-15.
90. Ye JS, Zheng XJ, Leung KW, Chen HM, Sheu FS. Induction of transient ion channel-like pores in a cancer cell by antibiotic peptide. J Biochem 2004;136(2):255-9.
91. Chen HM, Wang W, Smith D, Chan SC. Effects of the anti-bacterial peptide cecropin B and its analogs, cecropins B-1 and B-2, on liposomes, bacteria, and cancer cells. Biochim Biophys Acta 1997;1336(2):171-9.
92. Okumura K, Itoh A, Isogai E, Hirose K, Hosokawa Y, Abiko Y, et al. C-terminal domain of human CAP18 antimicrobial peptide induces apoptosis in oral squamous cell carcinoma SAS-H1 cells. Cancer Lett 2004;212(2):185-94.
93. Doyle J, Brinkworth CS, Wegener KL, Carver JA, Llewellyn LE, Olver IN, et al. nNOS inhibition, antimicrobial and anticancer activity of the amphibian skin peptide, citropin 1.1 and synthetic modifications. The solution structure of a modified citropin 1.1. Eur J Biochem 2003;270(6):1141-53.
94. Kim S, Kim SS, Bang YJ, Kim SJ, Lee BJ. In vitro activities of native and designed peptide antibiotics against drug sensitive and resistant tumor cell lines. Peptides 2003;24(7):945-53.
95. Lehmann J, Retz M, Sidhu SS, Suttmann H, Sell M, Paulsen F, et al. Antitumor activity of the antimicrobial peptide magainin II against bladder cancer cell lines. Eur Urol 2006;50(1):141-7.
96. Chen YQ, Min C, Sang M, Han YY, Ma X, Xue XQ, et al. A cationic amphiphilic peptide ABP-CM4 exhibits

selective cytotoxicity against leukemia cells. Peptides 2010;31(8):1504-10.
97. Riedl S, Rinner B, Asslaber M, Schaider H, Walzer S, Novak A, et al. In search of a novel target - phosphatidylserine exposed by non-apoptotic tumor cells and metastases of malignancies with poor treatment efficacy. Biochim Biophys Acta 2011;1808(11):2638-45.
98. Zachowski A. Phospholipids in animal eukaryotic membranes: transverse asymmetry and movement. Biochem J 1993;294 (Pt 1)(Pt 1):1-14.
99. Risso A, Braidot E, Sordano MC, Vianello A, Macrì F, Skerlavaj B, et al. BMAP-28, an antibiotic peptide of innate immunity, induces cell death through opening of the mitochondrial permeability transition pore. Mol Cell Biol 2002;22(6):1926-35.
100. Berge G, Eliassen LT, Camilio KA, Bartnes K, Sveinbjørnsson B, Rekdal O. Therapeutic vaccination against a murine lymphoma by intratumoral injection of a cationic anticancer peptide. Cancer Immunol Immunother 2010;59(8):1285-94.
101. Oršolić N, Josipović P, Bašić IJEJoNT. Direct antitumor activity of honey bee venom in vivo and in vitro. Egyptian Journal of Natural Toxins 2009;6(1):1-15.
102. Wu SP, Huang TC, Lin CC, Hui CF, Lin CH, Chen JY. Pardaxin, a fish antimicrobial peptide, exhibits antitumor activity toward murine fibrosarcoma in vitro and in vivo. Mar Drugs 2012;10(8):1852-72.
103. Azevedo RA, Ferreira AK, Auada AVV, Pasqualoto KFM, Marques-Porto R, Maria DA, et al. Antitumor effect of cationic INKKI peptide from bovine β-casein on melanoma B16F10. J Canc Ther 2012.
104. Chen YL, Li JH, Yu CY, Lin CJ, Chiu PH, Chen PW, et al. Novel cationic antimicrobial peptide GW-H1 induced caspase-dependent apoptosis of hepatocellular carcinoma cell lines. Peptides 2012;36(2):257-65.
105. Rodrigues EG, Dobroff AS, Cavarsan CF, Paschoalin T, Nimrichter L, Mortara RA, et al. Effective topical treatment of subcutaneous murine B16F10-Nex2 melanoma by the antimicrobial peptide gomesin. Neoplasia 2008;10(1):61-8.
106. Hilchie AL, Doucette CD, Pinto DM, Patrzykat A, Douglas S, Hoskin DW. Pleurocidin-family cationic antimicrobial peptides are cytolytic for breast carcinoma cells and prevent growth of tumor xenografts. Breast Cancer Res 2011;13(5):R102.
107. Liu S, Yang H, Wan L, Cai HW, Li SF, Li YP, et al. Enhancement of cytotoxicity of antimicrobial peptide magainin II in tumor cells by bombesin-targeted delivery. Acta Pharmacol Sin 2011;32(1):79-88.
108. Winder D, Günzburg WH, Erfle V, Salmons B. Expression of antimicrobial peptides has an antitumour effect in human cells. Biochem Biophys Res Commun 1998;242(3):608-12.
109. Russell PJ, Hewish D, Carter T, Sterling-Levis K, Ow K, Hattarki M, et al. Cytotoxic properties of immunoconjugates containing melittin-like peptide 101 against prostate cancer: in vitro and in vivo studies. Cancer Immunol Immunother 2004;53(5):411-21.
110. Hui L, Leung K, Chen HM. The combined effects of antibacterial peptide cecropin A and anti-cancer agents on leukemia cells. Anticancer Res 2002;22(5):2811-6.
111. Johnstone SA, Gelmon K, Mayer LD, Hancock RE, Bally MB. In vitro characterization of the anticancer activity of membrane-active cationic peptides. I. Peptide-mediated cytotoxicity and peptide-enhanced cytotoxic activity of doxorubicin against wild-type and p-glycoprotein over-expressing tumor cell lines. Anticancer Drug Des 2000;15(2):151-60.
112. Piotrowska U, Sobczak M, Oledzka E. Current state of a dual behaviour of antimicrobial peptides-Therapeutic agents and promising delivery vectors. Chem Biol Drug Des 2017;90(6):1079-93.
113. Fjell CD, Hiss JA, Hancock RE, Schneider G. Designing antimicrobial peptides: form follows function. Nat Rev Drug Discov 2011;11(1):37-51.
114. Meiller TF, Hube B, Schild L, Shirtliff ME, Scheper MA, Winkler R, et al. A novel immune evasion strategy of candida albicans: proteolytic cleavage of a salivary antimicrobial peptide. PLoS One 2009;4(4):e5039.
115. Roy H, Dare K, Ibba M. Adaptation of the bacterial membrane to changing environments using aminoacylated phospholipids. Mol Microbiol 2009;71(3):547-50.
116. Giménez D, Andreu C, del Olmo M, Varea T, Diaz D, Asensio G. The introduction of fluorine atoms or trifluoromethyl groups in short cationic peptides enhances their antimicrobial activity. Bioorg Med Chem 2006;14(20):6971-8.
117. Papo N, Shai Y. New lytic peptides based on the D,L-amphipathic helix motif preferentially kill tumor cells compared to normal cells. Biochemistry 2003;42(31):9346-54.
118. Svenson J, Stensen W, Brandsdal BO, Haug BE, Monrad J, Svendsen JS. Antimicrobial peptides with stability toward tryptic degradation. Biochemistry 2008;47(12):3777-88.
119. Knappe D, Henklein P, Hoffmann R, Hilpert K. Easy strategy to protect antimicrobial peptides from fast degradation in serum. Antimicrob Agents Chemother 2010;54(9):4003-5.
120. Arias M, Hilchie AL, Haney EF, Bolscher JG, Hyndman ME, Hancock RE, et al. Anticancer activities of bovine and human lactoferricin-derived peptides. Biochem Cell Biol 2017;95(1):91-8.
121. Ghosh C, Manjunath GB, Akkapeddi P, Yarlagadda V, Hoque J, Uppu DS, et al. Small molecular antibacterial peptoid mimics: the simpler the better! J Med Chem 2014;57(4):1428-36.
122. Wang Y, Yang YJ, Chen YN, Zhao HY, Zhang S. Computer-aided design, structural dynamics analysis, and in vitro susceptibility test of antibacterial peptides incorporating unnatural amino acids against microbial infections. Comput Methods Programs Biomed 2016;134:215-23.
123. de la Fuente-Núñez C, Reffuveille F, Haney EF, Straus SK, Hancock RE. Broad-spectrum anti-biofilm peptide that targets a cellular stress response. PLoS Pathog 2014;10(5):e1004152.

124. Lohan S, Cameotra SS, Bisht GS. Systematic study of non-natural short cationic lipopeptides as novel broad-spectrum antimicrobial agents. Chem Biol Drug Des 2013;82(5):557-66.
125. de Breij A, Riool M, Kwakman PH, de Boer L, Cordfunke RA, Drijfhout JW, et al. Prevention of Staphylococcus aureus biomaterial-associated infections using a polymer-lipid coating containing the antimicrobial peptide OP-145. J Control Release 2016;222:1-8.
126. McGee DC, Gould MK. Preventing complications of central venous catheterization. N Engl J Med 2003;348(12):1123-33.
127. Rijsbergen M, Rijneveld R, Todd M, Feiss GL, Kouwenhoven STP, Quint KD, et al. Results of phase 2 trials exploring the safety and efficacy of omiganan in patients with human papillomavirus-induced genital lesions. Br J Clin Pharmacol 2020;86(11):2133-43.
128. Lipsky BA, Holroyd KJ, Zasloff M. Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. Clin Infect Dis 2008;47(12):1537-45.
129. Cheng KT, Wu CL, Yip BS, Chih YH, Peng KL, Hsu SY, et al. The Interactions between the Antimicrobial Peptide P-113 and Living Candida albicans Cells Shed Light on Mechanisms of Antifungal Activity and Resistance. Int J Mol Sci 2020;21(7).
130. van der Does AM, Bogaards SJ, Ravensbergen B, Beekhuizen H, van Dissel JT, Nibbering PH. Antimicrobial peptide hLF1-11 directs granulocyte-macrophage colony-stimulating factor-driven monocyte differentiation toward macrophages with enhanced recognition and clearance of pathogens. Antimicrob Agents Chemother 2010;54(2):811-6.
131. Sveinbjørnsson B, Camilio KA, Haug BE, Rekdal Ø. LTX-315: a first-in-class oncolytic peptide that reprograms the tumor microenvironment. Future Med Chem 2017;9(12):1339-44.
132. Spicer J, Marabelle A, Baurain JF, Jebsen NL, Jøssang DE, Awada A, et al. Safety, Antitumor Activity, and T-cell Responses in a Dose-Ranging Phase I Trial of the Oncolytic Peptide LTX-315 in Patients with Solid Tumors. Clin Cancer Res 2021.

AUTOLOGOUS CARTILAGE REPAIR WITH AUTOCART: A CASE REPORT OF KNEE OSTEOCHONDRAL LESION TREATMENT

Zorica Vangelovska[1], Zoran Nestorovski[1], Ana-Marija Maneva[2], Goran Vrgoc[3] and Aleksandar Matic[4'5]

[1]*General Hospital "8th of September", Department of Orthopedics and Traumatology, Skopje, North Macedonia*
[2] *PHI Clinical Hospital- Shtip, Department of Orthopedics and Traumatology , Shtip, North Macedonia*
[3]*University Hospital Sveti Duh, Department of Orthopedic surgery , Zagreb, Croatia*
[4] *University of Kragujevac, Kragujevac, Faculty of Medical Sciences, Department of Surgery, Serbia*
[5] *University Clinical Center, Department of Orthopedics and Traumatology, Kragujevac,Serbia*

Received: 15.12.2025.
Accepted: 24.03.2026.

Corresponding author:

Zorica Vangelovska

General Hospital "8th of September", Department of Orthopedics and Traumatology, Skopje, North Macedonia

E-mail: vangelovskaz@gmail.com

ABSTRACT

Osteochondral lesions of the knee represent a challenging pathology due to their limited intrinsic healing potential. These defects are frequently encountered in young and active individuals and may lead to persistent pain, functional impairment, and progression to early osteoarthritis if left untreated. Conventional surgical strategies, such as microfracture, autologous chondrocyte implantation (ACI) and osteochondral transfer procedures, have shown variable outcomes and are often associated with increased morbidity, prolonged rehabilitation or the need for staged interventions. The AutoCart procedure, a single-stage arthroscopic minced cartilage technique, combines autologous cartilage fragments with platelet-rich plasma and autologous thrombin. This approach provides a biologically favorable environment for cartilage repair while reducing the complexity, cost and regulatory demands associated with multi-stage procedures.

Keywords: *AutoCart procedure, arthroscopy, cartilage repair,osteochondral lesion.*

UDK:

Eabr 2026; 27(2):199-204
DOI:10.2478/eabr-2026-0019

INTRODUCTION

The true incidence of osteochondral lesions in humans remains uncertain, as a substantial proportion of cases remain asymptomatic.(1,2) Articular cartilage damage leading to chondral lesions may result from metabolic, genetic, vascular, or traumatic factors. These defects may develop after a single episode of excessive joint loading or as a consequence of repetitive microtrauma over time. Depending on the extent of cartilage involvement, lesions may range from small superficial defects to osteochondral injuries affecting the full thickness of the cartilage and the underlying subchondral bone.(3)Although the exact prevalence of cartilage lesions is difficult to determine, several studies have reported that articular cartilage defects are identified in approximately 60-66% of knees undergoing arthroscopic evaluation.(4) The management of focal chondral injuries continues to represent a significant challenge for orthopedic surgeons. The primary goals of treatment are pain reduction, restoration of joint function, and facilitation of a return to normal daily activities and an active lifestyle.Therapeutic strategies include conservative management, palliative procedures, and reparative or restorative surgical techniques aimed at promoting articular cartilage regeneration. Commonly utilized surgical procedures include microfracture, autologous chondrocyte implantation (ACI), osteochondral autologous transfer (OATS), and minced cartilage implantation (MCI). The concept of minced cartilage implantation was first introduced in 1983.(5)In recent years, increasing attention has been directed toward single-stage cartilage repair techniques that avoid the need for in vitro cell expansion while preserving viable chondrocytes capable of producing extracellular matrix and supporting cartilage regeneration.(6,7) Contemporary clinical studies have demonstrated encouraging short- and mid-term outcomes following arthroscopic autologous minced cartilage implantation for focal cartilage defects of the knee.(8)Furthermore, biologically augmented approaches combining minced cartilage fragments with platelet-rich plasma or fibrin-based matrices have been proposed to enhance graft stability and create a more favorable biological environment for cartilage repair.(9) These developments have contributed to the emergence of standardized arthroscopic techniques that allow simultaneous harvesting and implantation of autologous cartilage fragments within a single surgical procedure.The aim of this case report is to present the clinical and radiological outcome of a single-stage arthroscopic treatment of a focal cartilage lesion of the lateral femoral condyle in a young male patient using a biologically augmented minced cartilage technique.

CASE REPORT

A 23-year-old male patient presented to the orthopedic clinic with complaints of pain localized to the left knee. According to his medical history, the pain had been present for approximately one year, with progressive worsening over the preceding several months. The patient denied any history of trauma. On physical examination, the range of motion was within normal limits, although pain occurred at maximum flexion. Clinical tests, including McMurray, Lachman and Ballottement were negative. Radiological evaluation with plain radiographs and MRI revealed an osteochondral lesion located at the lateral femoral condyle and loose body. Based on these findings, surgical management was indicated and the patient was scheduled for knee arthroscopy with an AutoCart procedure.

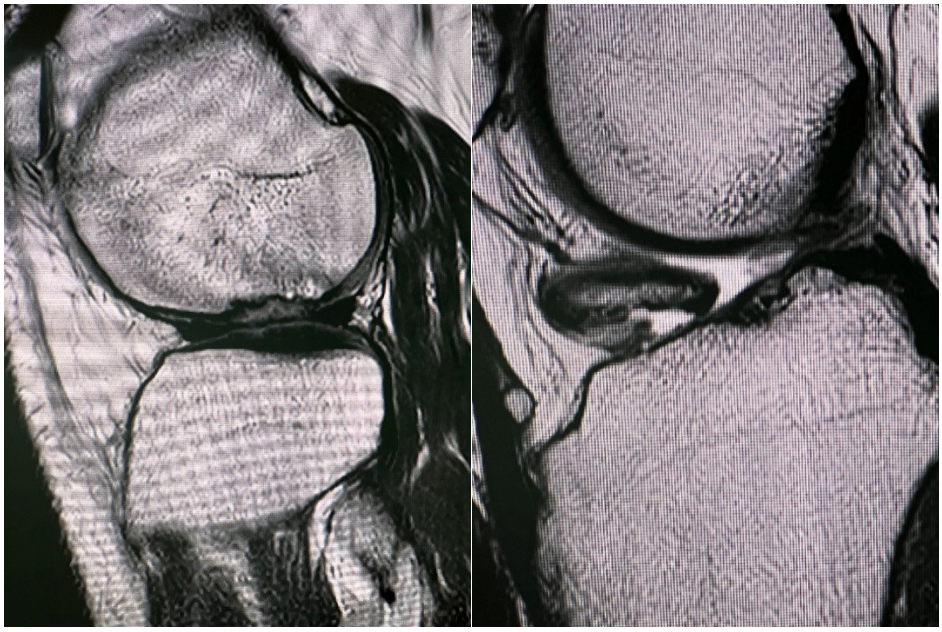


Figure 1. Preoperative MRI showing an osteochodral lesion of the lateral femoral condyle and intraarticular loose body

Under spinal anesthesia, arthroscopy of the left knee was performed using two standard portals. An osteochondral lesion was identified on the lateral femoral condyle. In addition, a large intra-articular loose body was visualized and subsequently removed.A 5-mm shaver blade was then used to harvest autologous chondral fragments from the healthy cartilage margins surrounding the lesion. A sterile tissue-collection device connected to suction was used to allow simultaneous harvesting and collection of viable cartilage fragments for subsequent implantation. Once harvesting was completed, the tissue collector was opened and the filter chamber containing the cartilage fragments was retrieved. A total of 15 mL of venous blood was aspirated using a double-syringe system for platelet-rich plasma (PRP) preparation and centrifuged at 1500 rpm for 5 minutes. The resulting plasma was transferred into a smaller syringe. Preparation of autologous thrombin was subsequently performed using a PRP-based activation system according to the manufacturer's protocol. The plasma was sequentially processed and allowed to incubate in a horizontal position to facilitate thrombin generation, after which it was aspirated into a sterile syringe for intraoperative use.Using a connector system, the harvested chondral fragments were mixed with PRP in a 3:1 ratio. Through several forward-and-back transfers between syringes, a homogeneous paste-like composite was created and loaded into a 1-mL syringe equipped with an application cannula.After evacuation of intra-articular fluid, the osteochondral defect was carefully dried and the arthroscopic trocar was withdrawn to create a dry working environment. The prepared chondral-PRP composite was then applied directly onto the lesion site. Autologous thrombin was subsequently applied to promote graft stabilization and biological fixation within the defect. Care was taken to ensure uniform distribution and stable adherence of the graft material to the surrounding cartilage margins.

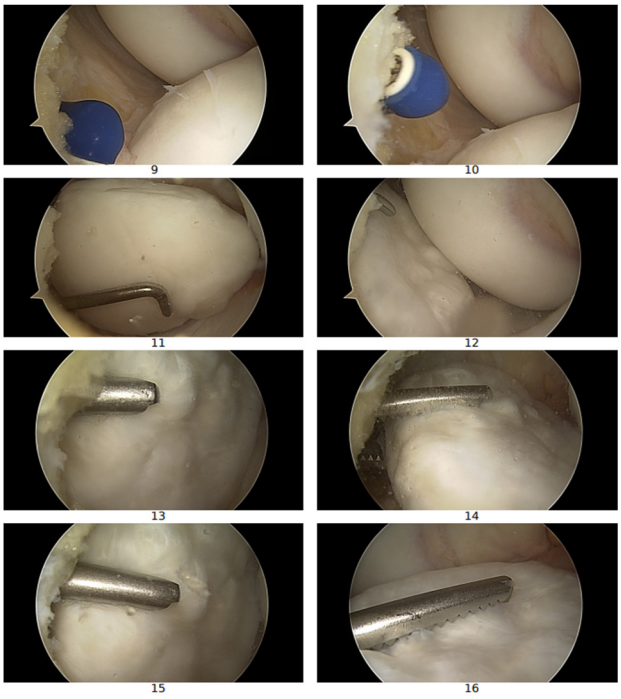


Figure 2. Arthroscopic view of intaarticular loose body

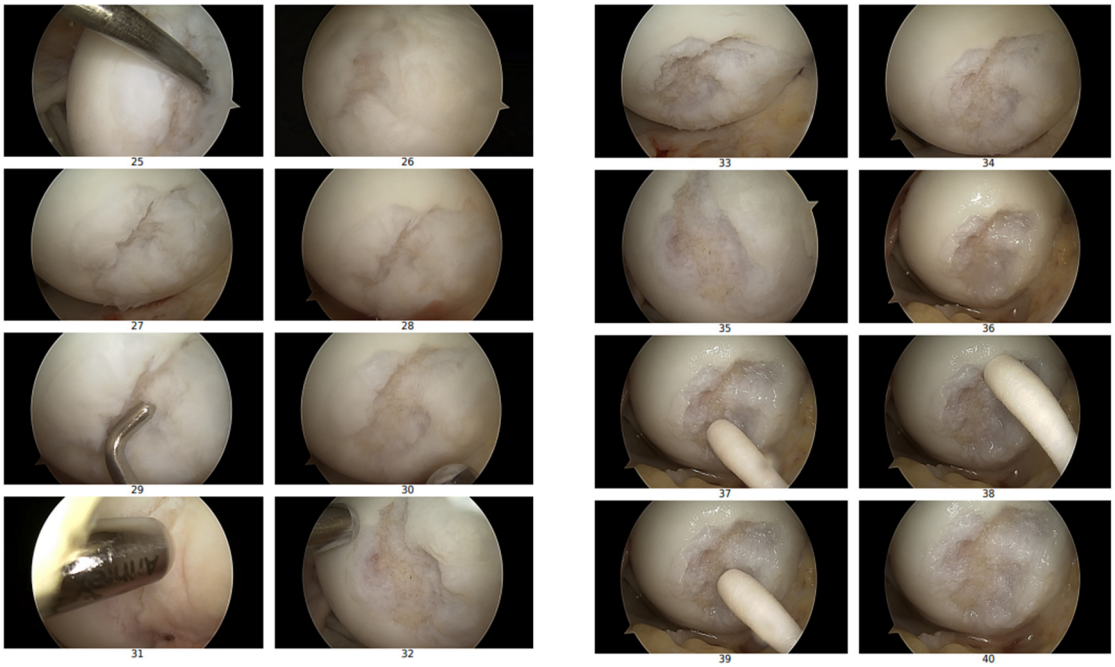


Figure 3. Arthroscopic view of an osteochondral lesion of the lateral femoral condyle

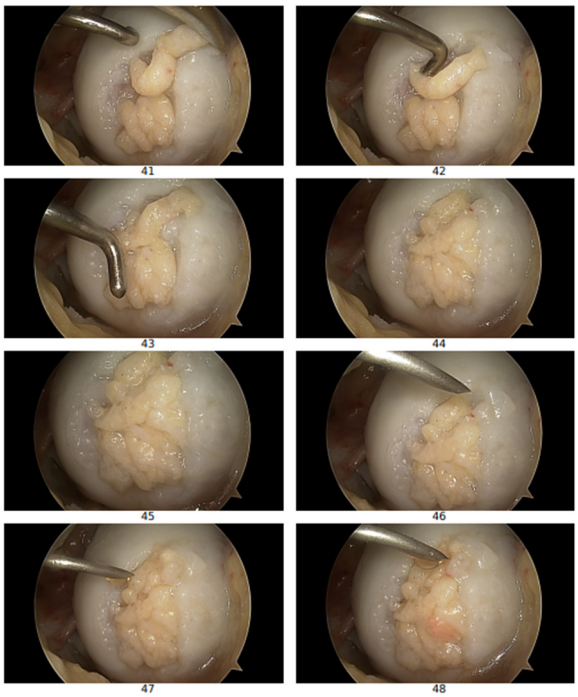


Figure 4. Arthroscopic images showing the osteochondral lesion after filling with the minced cartilage-PRP composite during the AutoCart procedure with uniform graft distribution

In the immediate postoperative period, early mobilization and structured physical rehabilitation were initiated. Thromboprophylaxis was administered with rivaroxaban 10 mg once daily for two weeks. The patient was instructed to ambulate with two axillary crutches while maintaining a non-weight-bearing regimen during the initial rehabilitation phase.At 24-month follow-up, clinical examination demonstrated full range of motion without joint effusion, instability or mechanical symptoms. Follow-up evaluations were performed at 6, 12, and 24 months postoperatively. Preoperatively, the Visual Analog Scale (VAS) pain score was 6, the International Knee Documentation Committee (IKDC) subjective score was 45, and the Tegner activity level was 3. At 12-month follow-up, substantial clinical improvement was observed, with VAS decreasing to 0, IKDC increasing to 82, and Tegner activity level improving to 5. At 24-month follow-up, the outcome remained stable, with VAS remaining 0, IKDC further improving to 90 and Tegner activity level maintained at 5, indicating sustained functional recovery and complete resolution of pain.

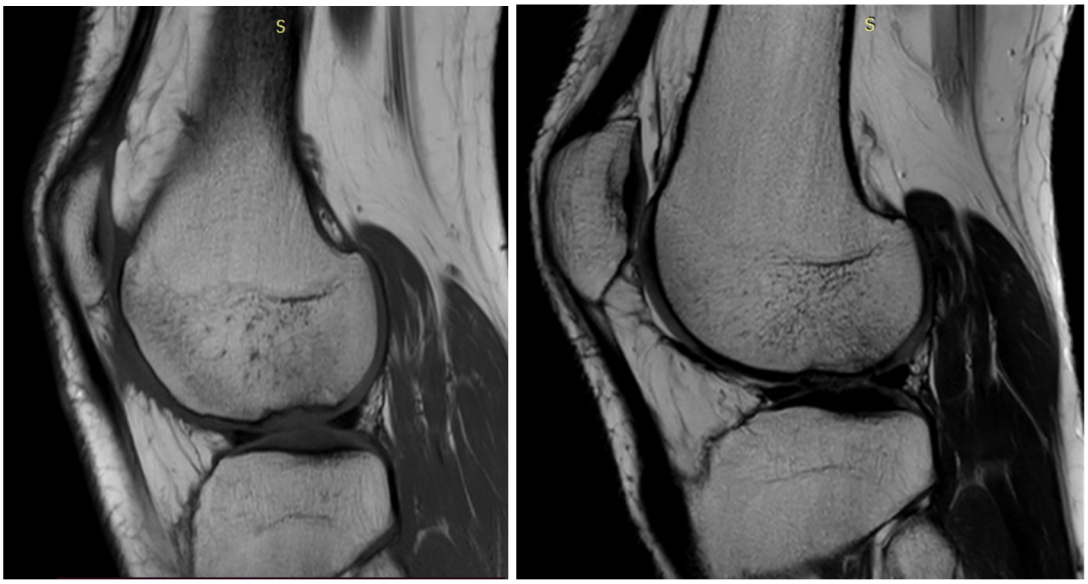


Figure 5. MRI of the left Knee:12 and 24 months postoperatively

DISCUSSION

The present case demonstrated favorable clinical and radiological improvement following a fully arthroscopic autologous minced cartilage implantation using the AutoCart technique for the treatment of a focal osteochondral lesion of the knee. The encouraging outcome observed in this patient supports the growing interest in single-stage biological cartilage repair strategies that aim to restore joint surface integrity while preserving the biological potential of autologous chondrocytes. Articular cartilage lesions may arise from traumatic injury, degenerative processes, dysplasia, or osteochondritis dissecans. In physically active individuals, repetitive mechanical loading may compromise subchondral bone circulation, which may predispose the joint to progressive cartilage degeneration. If left untreated, focal cartilage defects may

eventually lead to the development of degenerative osteoarthritis. Therefore, surgical intervention is frequently required to restore the articular surface and stimulate regeneration of cartilage tissue resembling native hyaline cartilage.(9) Over the past two decades, several cartilage repair strategies have been developed, including microfracture, osteochondral autograft transplantation, autologous chondrocyte implantation (ACI), and more recently, minced cartilage implantation techniques. One of the early clinical experiences with autologous cartilage repair was reported by Stone et al., who demonstrated significant clinical improvement in patients with Outerbridge grade IV cartilage lesions treated using autologous cartilage repair techniques.(10)Subsequent studies have explored biologically augmented approaches combining cartilage fragments with autologous bone or biological scaffolds. Christensen et al. described the use of autologous dual-tissue transplantation (ADTT), which combines autologous cartilage and bone fragments for the treatment of osteochondral lesions. Their results demonstrated significant improvement in clinical outcomes and restoration of subchondral bone structure at one-year follow-up.(11) More recently, single-stage minced cartilage implantation has gained increasing attention as a biologically attractive alternative to traditional cartilage repair techniques. Massen et al. reported significant improvements in clinical scores and patient-reported outcome measures in patients treated with autologous minced cartilage implantation at two-year follow-up. Importantly, minced cartilage fragments preserve viable chondrocytes capable of producing extracellular matrix and contributing to hyaline-like cartilage repair tissue.(12) Similarly, Runer et al. demonstrated favorable mid-term outcomes in patients treated with minced cartilage implantation, with high graft durability observed at 60 months of follow-up and a very low revision rate.(13) Focal chondral lesions of the knee represent a significant clinical challenge due to the limited intrinsic healing capacity of articular cartilage and the potential progression toward early osteoarthritis if left untreated (14). Blanke et al. further reported significant improvements in functional knee scores and satisfactory defect filling on MRI in patients treated with all-autologous minced cartilage procedures (15). Comparable improvements in patient-reported outcomes were also observed in the study by Schneider et al., confirming the clinical effectiveness of this biological cartilage repair technique (7). Regenerative cartilage repair strategies have evolved considerably in recent years, with various biological procedures demonstrating encouraging clinical and functional outcomes (16-19). These approaches aim to restore the articular surface and improve joint function, particularly in young and active patients with symptomatic cartilage defects. Pohl et al. reported encouraging clinical and radiological outcomes two years after autologous shaver-based minced cartilage implantation for cartilage lesions of the knee. Their findings demonstrated significant improvement in clinical scores and MRI parameters, highlighting the regenerative potential of minced cartilage techniques and their effectiveness as a single-stage cartilage repair strategy.(20) In addition, Başal et al. evaluated autologous minced cartilage implantation combined with an arthroscopic platelet-rich fibrin membrane for the treatment of large chondral defects of the knee. Their prospective comparative study demonstrated significant clinical improvement and satisfactory defect filling on follow-up imaging, suggesting that biological augmentation may further enhance cartilage regeneration and improve treatment outcomes.(21) Compared with conventional autologous chondrocyte implantation (ACI), the AutoCart technique offers several potential advantages. It represents a single-stage procedure that avoids the need for in vitro cell expansion and a second surgical intervention. This reduces treatment costs, regulatory complexity, and overall rehabilitation time. Furthermore, the procedure can be performed arthroscopically, allowing for a minimally invasive approach while preserving the biological properties of autologous cartilage fragments. Despite these encouraging findings, several limitations must be acknowledged. First, the present report describes a single clinical case, which limits the generalizability of the results. Second, although clinical improvement and favorable radiological findings were observed, standardized patient-reported outcome scores such as IKDC, KOOS, or Lysholm were not prospectively recorded. Third, longer follow-up is necessary to determine the durability of the repair tissue and to evaluate the long-term clinical outcomes of the AutoCart technique. Nevertheless, the present case contributes to the growing body of evidence supporting single-stage biological cartilage repair strategies. The favorable clinical course and radiological improvement observed in this patient suggest that arthroscopic autologous minced cartilage implantation using the AutoCart technique may represent a promising treatment option for focal osteochondral lesions of the knee. However, larger prospective studies with standardized outcome measures and longer follow-up are required to confirm the clinical effectiveness and long-term durability of this technique.

CONCLUSION

This case demonstrates the feasibility of the AutoCart procedure as a fully arthroscopic, minimally invasive single-stage technique for the treatment of focal osteochondral lesions of the knee. By utilizing autologous cartilage fragments combined with biological augmentation, the technique may create a favorable biological environment that supports cartilage repair and restoration of the articular surface.

In the present patient, encouraging clinical and radiological improvement was observed during short-term follow-up. However, as this report describes a single clinical case, the findings should be interpreted with caution. Further prospective studies involving larger patient cohorts, standardized clinical outcome measures, and longer follow-up periods are necessary to better define the role and long-term effectiveness of the AutoCart technique in the treatment of osteochondral defects of the knee.

REFERENCES

1. Curl WW, Krome J, Gordon ES, Rushing J, Smith BP, Poehling GG. Cartilage injuries: a review of 31,516 knee arthroscopies. *Arthroscopy*. 1997;13(4):456-460. doi:10.1016/S0749-8063(97)90124-9.
2. Widuchowski W, Widuchowski J, Trzaska T. Articular cartilage defects: study of 25,124 knee arthroscopies. *Knee*. 2007;14(3):177-182. doi:10.1016/j.knee.2007.02.001
3. Cavalcanti FMMC, Doca D, Cohen M, Ferretti M. Updating on diagnosis and treatment of chondral lesions of the knee. *Rev Bras Ortop*. 2015;47(1):12-20. doi:10.1016/S2255-4971(15)30339-6
4. Merkely G, Ackermann J, Lattermann C. Articular cartilage defects: incidence, diagnosis, and natural history. *Oper Tech Sports Med*. 2018;26(3):156-162. doi:10.1053/j.otsm.2018.06.008
5. Albrecht F, Roessner A, Zimmermann E. Closure of osteochondral lesions using chondral fragments and fibrin adhesive. *Arch Orthop Trauma Surg*. 1983;101(3):213-217.
6. Runer A, Ossendorff R, Öttl F, Kogler F, Reider S, Salzmann GM. Autologous minced cartilage repair for chondral and osteochondral lesions of the knee joint demonstrates good postoperative outcomes and low reoperation rates at minimum five-year follow-up. *Knee Surg Sports Traumatol Arthrosc.* 2023;31(12):4977-4987. doi:10.1007/s00167-023-07531-0.
7. Schneider S, Ossendorff R, Walter SG, Berger M, Endler C, Kaiser R, Ilg A, Salzmann GM, Holz J. Arthroscopic autologous minced cartilage implantation of cartilage defects in the knee: a 2-year follow-up of 62 patients. *Orthop J Sports Med.* 2024;12(12):23259671241297970. doi:10.1177/23259671241297970.
8. Pohl S, Jung C, Gesslein M, et al. Clinical and radiological outcomes after autologous shaver-based minced cartilage implantation for cartilage lesions of the knee. *Arch Orthop Trauma Surg.* 2025. doi:10.1007/s00402-025-05441-4.
9. Barbaret A, Wein F, Jacquet C, et al. One-stage minced cartilage autograft with platelet-rich plasma for knee cartilage defects. *J Exp Orthop.* 2025;12:62. doi:10.1002/jeo2.70162.
10. Stone KR, Walgenbach AW, Freyer A, Turek TJ, Speer DP. Articular cartilage paste grafting to full-thickness knee joint lesions: 2- to 12-year follow-up. *Arthroscopy*. 2006;22(3):291-299. doi:10.1016/j.arthro.2005.12.051
11. Christensen BB, Foldager CB, Jensen J, Lind M. Autologous dual-tissue transplantation for osteochondral repair: early clinical and radiological results. *Cartilage*. 2015;6(3):166-173. doi:10.1177/1947603515580983
12. Massen FK, Inauen CR, Harder LP, Runer A, Preiss S, Salzmann GM. One-step autologous minced cartilage procedure for osteochondral lesions: a series of 27 patients with 2-year follow-up. *Orthop J Sports Med*. 2019;7(6):2325967119853773. doi:10.1177/2325967119 853773.
13. Runer A, Ossendorff R, Öttl F, et al. Autologous minced cartilage repair for chondral and osteochondral lesions of the knee joint demonstrates good postoperative outcomes and low reoperation rates at minimum five-year follow-up. *Knee Surg Sports Traumatol Arthrosc*. 2023;31(12):4977-4987. doi:10.1007/s00167-023-07531-0.
14. Seo SS, Kim CW, Jung DW. Management of focal chondral lesion in the knee joint. *Knee Surg Relat Res*. 2011;23(4):185-196. doi:10.5792/ksrr.2011.23.4.185.
15. Blanke F, Warth F, Oehler N, Siegl J, Prall WC. Autologous platelet-rich plasma and fibrin-augmented minced cartilage implantation in chondral lesions of the knee leads to good clinical and radiological outcomes after more than 12 months: a retrospective cohort study of 71 patients. *J Exp Orthop*. 2024;11(4):e70051. doi:10.1002/jeo2.70051
16. Siebold R, Dehler C, Ellermann A, et al**.** Combined arthroscopic meniscal allograft transplantation and autologous chondrocyte implantation for treatment of meniscal insufficiency with concomitant full-thickness cartilage defects: medium-term clinical outcomes. *Arthroscopy.* 2023;39(6):1493-1503. doi:10.1016/j.arthro.2023.01.028
17. Proffen B, von Keudell A, Vavken P. Evidence-based therapy for cartilage lesions in the knee: regenerative treatment options. *Z Orthop Unfall*. 2012;150(3):280-289. doi:10.1055/s-0031-1298387.
18. Valtanen RS, Arshi A, Kelley BV, Fabricant PD, Jones KJ. Articular cartilage repair of the pediatric and adolescent knee with regard to minimal clinically important difference: a systematic review. *Cartilage*. 2018;10(4):389-399. doi:10.1177/1947603518783503.
19. Schindler OS. Cartilage repair using autologous chondrocyte implantation techniques. *J Perioper Pract*. 2009;19(2):60-64. doi:10.1177/175045890901900203
20. Pohl S, Mühler M, Zimmerer A, Schoon J, Wassilew GI, Gebhardt S. Clinical and radiological 2-year results after autologous shaver-based minced cartilage implantation for cartilage lesions of the knee. *Arch Orthop Trauma Surg*. 2025. doi:10.1007/s00402-025-06010-8.
21. Başal Ö, Jefferies JG, Serdar J, Doral MN. Autologous minced cartilage implantation combined with arthroscopic platelet-rich fibrin membrane in large chondral defects of the knee: a prospective comparative study. *J Cartilage Joint Preserv*. 2025. doi:10.1016/j.jcjp.2025.100270.

MANAGEMENT OF PULP CANAL OBLITERATION IN A TRAUMATIZED MAXILLARY INCISOR USING CONE-BEAM COMPUTED TOMOGRAPHY: A CASE REPORT

Kristina Krstic[1†], Nevena Cvetic[1†], Andjelka Simic[1], Jovan Rakic[1], Milos *Papic*[1]* and Suzana Zivanovic[1]

[1]*University of Kragujevac, Faculty of Medical Sciences, Department of Dentistry, Kragujevac, Serbia*

[†]Kristina Krstic and Nevena Cvetic contributed equally to this work and both should be considered first author.

Received: 05.12.2025.
Accepted: 29.04.2026.

Corresponding author:

Milos Papic

University of Kragujevac, Serbia, Faculty of Medical Sciences, Department of Dentistry, 69 Svetozara Markovica Street, 34000 Kragujevac, Serbia

Telephone: +381 (0) 34 306 800

E-mail: milos.papic@fmn.kg.ac.rs

ABSTRACT

Pulp canal obliteration (PCO), a common consequence of dental trauma, complicates endodontic treatment due to canal calcification and altered internal anatomy. This case report describes the diagnosis and management of a maxillary right central incisor affected by PCO in a 17-year-old female patient with a history of dental trauma five years prior. Clinical and radiographic examination revealed crown discoloration, a non-vital pulp, and absence of a visible root canal on periapical radiograph. Cone-beam computed tomography (CBCT) was employed to provide a three-dimensional evaluation, revealing canal obliteration with a patent segment in the apical third. Endodontic treatment was performed over two sessions under rubber dam isolation using magnification and ultrasonic-assisted irrigation. The canal was negotiated with small hand K-files (#6–15), the working length determined at 22 mm using an apex locator, and instrumentation completed with the RC Blue system (size 25/08) in reciprocal motion. The patient was asymptomatic postoperatively, and instructions were provided with a subsequent appointment scheduled for definitive restoration. This case underscores the importance of CBCT and modern endodontic techniques in managing complex calcified canals safely and effectively.

Keywords: *Pulp canal obliteration, dental trauma, CBCT, root canal treatment.*

UDK:

Eabr 2026; 27(2):205-210
DOI:10.2478/eabr-2026-0020

INTRODUCTION

Pulp canal obliteration (PCO) represents progressive deposition of hard tissue within the pulp chamber and root canal (1). PCO is referred to as calcific metamorphosis or pulp calcification (2). The formation of calcified tissue within the pulp space reflects a complex biological reaction of odontoblasts and subodontoblastic cells (3). As a consequence of calcification, the canal lumen becomes partially or completely obliterated by mineralized material resembling tertiary dentin (4). PCO may occur as part of physiological aging or as a response to external irritation, such as caries, restorative procedures, orthodontic forces, or dental trauma (5). Among these, trauma is one of the most recognized predisposing factors, since the disruption of pulpal blood supply following luxation or concussion injuries often triggers accelerated dentin deposition during the reparative phase. The degree of obliteration depends on the severity of the initial trauma and the pulp's reparative capacity, and may progress gradually over months or years (6).

When endodontic therapy becomes necessary, the absence of a visible canal greatly complicates the procedure. Locating the canal orifice and establishing a glide path are particularly challenging, often requiring the application of modern endodontic therapeutic approaches (7). Excessive removal of coronal dentin during the search for the canal can compromise tooth integrity and increase the risk of perforation or instrument fracture (5). Even when the canal is successfully negotiated, adequate cleaning and shaping may be compromised due to irregular canal geometry and reduced accessibility (8). In trauma-related cases, these challenges are even more pronounced. Traumatized teeth often exhibit not only calcific changes but also crown discoloration, altered dentin morphology, and apical resorption, further complicating diagnosis and treatment (2, 9). Because obliteration develops gradually, it is often diagnosed incidentally at an advanced stage, when the canal is already completely calcified and clinical symptoms such as pulpitis or a periapical lesion have appeared (10).

Diagnosis requires a combination of clinical evaluation and high-resolution imaging, as sensibility tests often yield false-negative results, and conventional radiographs may fail to depict the early stages of calcification (6). Two-dimensional radiography has limited diagnostic accuracy in detecting obliteration, especially in anterior teeth, where overlapping structures obscure subtle changes within the root canal system (4). Advanced imaging modalities, such as cone-beam computed tomography (CBCT) provide three-dimensional visualization that enables detection of residual canal space, assessment of the extent and orientation of calcification, and identification of periapical pathology (11). Furthermore, the combination of CBCT and 3D printing has emerged as a state-of-the-art option for canal localization in severely calcified teeth, although its use is currently restricted to specialized clinical and research settings (12). Nevertheless, in everyday practice, diagnosis of pulp canal obliteration still relies primarily on conventional radiography and clinical assessment, with CBCT reserved for complex or unclear cases (13).

The aim of this case report is to evaluate the role of CBCT in the diagnosis and treatment planning of teeth with root canal obliteration, particularly those resulting from trauma, and to emphasize the diagnostic and therapeutic challenges this condition poses in clinical endodontic practice.

CASE REPORT

A 17-year-old female patient, with no reported systemic medical conditions, presented to the Department of Dentistry, Faculty of Medical Sciences, complaining of sensitivity in the region of the maxillary right central incisor. Her dental history revealed a traumatic injury to the same tooth five years prior, for which no treatment had been undertaken. The patient reported occasional dull pain and sensitivity to thermal stimuli. Clinical examination revealed discoloration of the crown of the affected tooth. Pulp vitality testing yielded a negative response, while percussion and palpation tests were unremarkable. Conventional radiography did not reveal any periapical pathology, and the root canal could not be visualized. Based on the patient's medical history, clinical examination, and additional diagnostic findings, a diagnosis of pulp necrosis was established for the maxillary right central incisor, indicating the need for endodontic treatment. The patient gave written informed consent before the treatment.

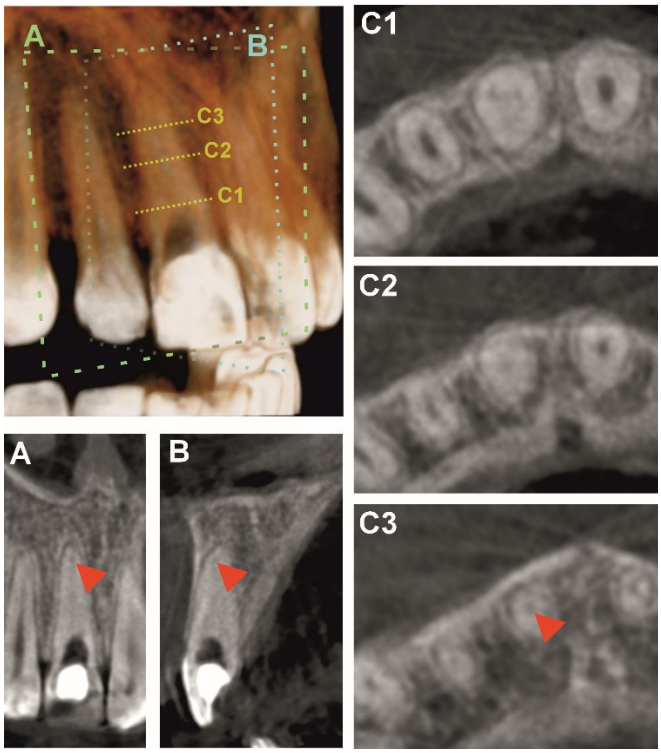
Endodontic treatment was performed in two sessions. At the initial appointment, rubber dam (Cerkamed, Stalowa Wola, Poland) isolation was achieved, and an access cavity was prepared using a small round diamond bur under high-speed handpiece. Upon inspection, canal obliteration was noted and the canal orifice could not be located. Therefore, the procedure was postponed to a subsequent visit. For further evaluation, CBCT scan was obtained. The scanning was obtained following the ALARA (as low as reasonably achievable) principle using Orthophos XG 3D device (Sirona Dental Systems GmbH, Bensheim, Germany). The device was set for three-dimensional recording using VOLL HD (85 kV/6 mA, exposure time – 14.3 s) or VOLL HD2 settings (85 kV/10 mA, exposure time – 5.0 s) with a voxel size of 100 μm. CBCT imaging revealed extensive root canal obliteration, with the canal only discernible in the apical third (*Figure 1*). Additionally, a periapical radiolucency was observed after CBCT analysis. Based on these findings, the initial diagnosis of pulp necrosis was revised to chronic apical periodontitis affecting the maxillary right central incisor.

At the second appointment, an absolutely dry operative field was established, and magnification loupes (Illuco loupes 5,5x, Gunpo City, South Korea), were used to aid canal localization. With copious irrigation using 17% EDTA (MD-Cleanser EDTA Solution, Meta Biomed, Chugbuk, South Korea), 2% sodium hypochlorite (Chloraxid 2%, Cerkamed, Stalowa Wola, Poland), and saline, combined with ultrasonic activation, the canal orifice was successfully located. Initial canal negotiation was performed with a #6 K-

file, followed by progressive instrumentation using #8, #10, and #15 hand K-files (Dentac, Istanbul, Turkey). Working length was determined using an electronic apex locator (Woodpex III, Woodpecker, China), measuring 22 mm. Mechanical preparation was completed using the RC Blue rotary system (Dentsply Sirona, Charlotte, North Carolina, USA) in reciprocal motion. Chemomechanical preparation was achieved with a single 25/08 instrument using the crown-down technique and abundant ultrasonic-activated irrigation. Continuous irrigation with 2% sodium hypochlorite was maintained throughout instrumentation to prevent debris accumulation. Final irrigation was performed in the following sequence: 5.25% sodium hypochlorite, distilled water, 17% EDTA followed by distilled water, and a final rinse with 5.25% sodium hypochlorite and distilled water. This protocol ensured effective removal of the smear layer and chemical disinfection of the canal system.

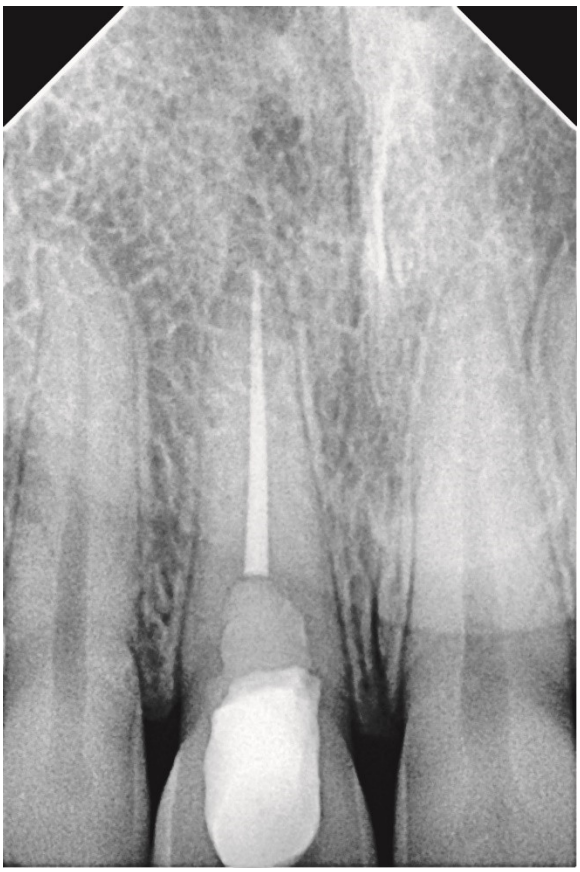
After completion of chemomechanical preparation, canal was dried with sterile paper points (Spident Co., Ltd., Incheon, South Korea) and obturated with cold lateral compaction technique and using Vio Seal (Spident Co., Ltd., Incheon, South Korea) sealer. The quality of the root canal filling was evaluated radiographically (Figure 2). The tooth was then temporarily restored, and postoperative instructions were provided. A subsequent visit was scheduled for placement of the definitive restoration.

Figure 1. A three-dimensional CBCT reconstruction illustrating the orientation of the imaging planes used for sectional analysis. The line labeled A represents the coronal plane, while B denotes the sagittal plane selected for detailed evaluation. The dotted markers C1, C2, and C3 indicate the axial slice levels corresponding to the coronal, middle, and apical thirds of the root canal, respectively.



The corresponding coronal (A) and sagittal (B) slices are shown in the lower left portion of the figure. The red arrowheads in these views indicate the location where the previously obstructed root canal becomes patent, marking the point of regained canal lumen continuity. The axial slices (C1–C3) displayed on the right depict cross-sectional morphology in the coronal, middle, and apical thirds of the canal, with the arrowhead in C3 highlighting the same transition point identified in the other planes.

Figure 2 - Retroalveolar radiograph after definitive root canal obturation



DISCUSSION

PCO represents a pathological process characterized by the gradual deposition of mineralized tissue within the pulp chamber and root canal system, frequently resulting in the radiographic loss of canal visibility (6). It may present as either total or partial obliteration. In cases of total calcification, the entire pulp canal system is radiographically indistinguishable, whereas partial obliteration is characterized by a reduced or partially calcified pulp chamber, with root canals still discernible but significantly narrowed (13).

The prevalence PCO has been widely documented in the endodontic literature, although results vary among studies. The incidental prevalence of PCO based on radiographic records has been estimated at 4.7% (14). However, the incidence increases substantially following dental trauma, ranging from 4% to 24% (6), which is consistent with our case where PCO developed after a traumatic injury to the maxillary right central incisor in a young patient. In teeth with immature root formation and who have experienced trauma, prevalence can be 56.9% (14). The prevalence of calcification is also found to be higher in anterior teeth, especially in central and lateral incisors (15). Obliteration occurs more frequently in individuals over 40 years of age, identifying age as a significant factor influencing the occurrence of PCO. In contrast, our case involved a 17-year-old patient, highlighting that PCO can also occur in younger individuals following trauma. Alamoudi *et al.*, in their case-control study, reported that the prevalence of pulp calcifications was higher in females (62%) compared to males (38%) (3). Although the exact etiology of pulpal calcification remains unclear (13), dental trauma is most commonly associated. Age has also been identified as a contributing factor (14). In addition, pulp canal obliteration may occur as a physiological response to chronic irritation, such as caries, deep restorations, trauma from occlusion, physiologic change, and orthodontic movement (7). Research have shown that the use of calcium hydroxide and hydrogen peroxide, can also promote calcification. Dentine dysplasia and long-term use of glucocorticoids have been correlated with calcification of the dental pulp (16).

While epidemiological studies have highlighted the prevalence and contributing factors of pulp canal obliteration, a thorough understanding of its clinical presentation and associated diagnostic challenges is crucial for effective patient management. Recognizing the subtle signs and limitations of conventional diagnostic methods allows clinicians to identify PCO early and implement appropriate treatment strategies. Diagnosing PCO is often challenging due to its complex clinical presentation and the limitations of conventional testing methods, making it essential to use an advanced diagnostic technique to accurately assess pulp status and canal morphology. PCO is most often discovered incidentally during routine radiographic examinations, as it typically progresses without evident clinical symptoms. Nevertheless, as the process advances, crown discoloration and pulp necrosis with periapical lesions and associated symptoms, may develop which represent the primary reasons patients seek dental treatment (10). This was evident in our case, where the patient presented with crown discoloration and sensitivity five years after trauma. Conventional pulp sensibility tests such as electric pulp testing and thermal tests (cold or heat) often yield unreliable or false-negative results. This occurs because these methods rely on neural stimulation, which may be reduced or absent in calcified canals, even when the pulp remains vital. Non-invasive diagnostic techniques such as pulse oximetry and laser Doppler flowmetry evaluate pulpal blood flow and have demonstrated higher accuracy in assessing pulp vitality. Consequently, clinicians should interpret negative sensibility test results in teeth with pulp canal obliteration cautiously and incorporate adjunctive vitality assessment methods when necessary (17).

According to the American Association of Endodontists, PCO is considered to be an endodontic case with a high degree of difficulty. Endodontic treatment is indicated only in the presence of acute symptoms and apical periodontitis, or when PCO complicates the treatment and adversely affects prognosis (18). In such cases, careful preoperative planning is essential. The most frequently reported difficulties include the extensive removal of tooth structure required during conventional access cavity preparation and the difficulty in locating the canal orifice (10). The access cavity preparation stage is a critical determinant of success in managing PCO, as it directly influences the clinician's ability to identify and negotiate the obliterated canal while minimizing procedural complications (10). The deposition of tertiary dentin in the pulp chamber results in an irregular and constricted canal anatomy, complicating access to the residual pulp tissue, particularly in the apical third of the root. These difficulties increase the risk of iatrogenic complications, such as perforations, ledge formation, loss of peri-cervical dentin and instrument fracture (4,19). Careful use of hand files, chelating agents such as 17% EDTA), and sodium hypochlorite irrigation has been shown to facilitate negotiation even in heavily calcified canals. In contrast, traditional methods, including the use of rigid stainless-steel hand instruments (K-files, C+ files), often prove inadequate in managing the complex and constricted anatomy of calcified canals. (4) Additionally, step-back preparation minimizes the risk of procedural errors, while rotary nickel–titanium (NiTi) systems have proven valuable for efficient mechanical shaping of the canal space (4). These challenges can be further mitigated by modern clinical aids such as CBCT, dental operating microscopy, and the use of ultrasonic tips to enhance precision and safety during treatment (10). In our case, canal negotiation was achieved using magnification loupes without the use of a dental operating microscope, which would have been the optimal method.

Preoperative radiographic evaluation is essential in cases of PCO to assess crown orientation, root curvature, and the degree of canal calcification. Conventional two-dimensional (2D) radiographs, while widely used, have inherent limitations due to superimposition of anatomical structures, geometric distortion, and restricted visualization of the three-dimensional anatomy of root canals (1). Canals that appear patent or calcified on 2D images may not accurately reflect clinical negotiability, and complete radiographic obliteration does not always indicate the absence of a canal space (13). By contrast, CBCT provides a three-dimensional evaluation of root canal anatomy, allowing precise assessment of canal morphology, orientation, extent of calcification, and residual canal space (6). As in our case, CBCT was used to confirm canal location and assess apical third negotiability, justifying its use given the complex obliteration. CBCT facilitates optimal access planning by estimating calcified tissue thickness and predicting the depth and path of the canal (13). However, CBCT is not intended to replace conventional radiography

but to complement it by providing additional diagnostic information (20). Its use requires specialized training and a thorough understanding of dental anatomy, as high-density structures can generate artifacts that may affect interpretation. CBCT also involves higher costs, limited availability, and exposure to ionizing radiation, so each scan must be justified by its potential diagnostic benefit. Image quality can be influenced by factors such as field of view, voxel size, number of projections, tube voltage, and the presence of materials within the canal (21). Clinical studies have demonstrated that the combination of CBCT, dental operating microscopy, and ultrasonic instrumentation improves canal negotiation, reduces procedural errors, and increases success rates in the treatment of PCO (1).

The present findings further highlight the clinical relevance of CBCT in the management of pulp canal obliteration, particularly in cases where conventional imaging assessment is limited. In accordance with the recommendations of the European Society of Endodontology, CBCT should be selectively utilized in complex endodontic situations, enhancing diagnostic precision and improving clinical outcomes (22). The present case supports existing evidence that CBCT can significantly reduce iatrogenic complications, improve canal localization, and increase the overall predictability of endodontic treatment in challenging PCO cases (23).

CONCLUSION

This case demonstrates that CBCT plays a crucial role in the diagnosis and management of teeth affected by pulp canal obliteration following trauma. Its ability to visualize root canal anatomy in three dimensions provides valuable information for determining the extent of calcification and planning a safe and conservative access pathway. The combination of CBCT imaging, the use of magnification, ultrasonic activation, and careful instrumentation enables successful negotiation and treatment of calcified canals while minimizing procedural complications. Early and accurate diagnosis supported by advanced imaging techniques can significantly improve the prognosis and long-term outcomes of endodontic treatment in such complex cases. This case highlights the importance of integrating advanced imaging and meticulous endodontic techniques in managing complex calcified teeth.

ACKNOWLEDGMENTS

This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, agreement No. 451-03-47/2023-01/200111, and the Faculty of Medical Sciences, University of Kragujevac, Serbia, project number JP 07-25.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

1. Braga Diniz JM, Diniz Oliveira HF, Pinto Coelho RC, Manzi F, Silva FE, Carvalho Machado V, Ribeiro Sobrinho AP, Fonseca Tavares WL. Guided Endodontic Approach in Teeth with Pulp Canal Obliteration and Previous Iatrogenic Deviation: A Case Series. Iran Endod J. 2022;17(2):78-84.
2. Nabavi S, Navabi S, Mohammadi SM. Management of Pulp Canal Obliteration in Mandibular Incisors with Guided Endodontic Treatment: A Case Report. Iran Endod J. 2022;17(4):216-219.
3. Jadhav GR, Mittal P. Cone-Beam Computed Tomographic Scan-based Assessment of the Correlation between the Location of Caries and Pulp Canal Obliteration: An Aid to Treatment Planning. J Endod. 2025;51(1):15-20.
4. Giri K, Banga K, Arora S, Elmsmari F, Pawar AM. Management of calcified canals during root canal treatment: A systematic review of case reports. PeerJ. 2025;13:e19900.
5. Valverde Haro HP, Quille Punina LG, Erazo Conde AD. Guided Endodontic Treatment of Mandibular Incisor with Pulp Canal Obliteration following Dental Trauma: A Case Report. Iran Endod J. 2024;19(3):223-227.
6. Liu H, Shen Y. Cone-beam computed tomography-guided freehand technique for managing pulp canal obliteration: Clinical tips and precautions. J Dent Sci. 2025;20(4):2497-2499.
7. Lewis NV, Aggarwal S. Static Guided Endodontic Approach for Pulp Canal Obliteration: A Case Report. Cureus. 2023;15(7):e42379.
8. Kasabwala KA, Saumya-Rajesh P, Velmurugan N, Ashritha M. Pulp Canal Obliteration: A Review. J Oper Dent Endod. 2020;5(1):6-11.
9. Kamburoğlu K, Sönmez G, Koç C, Yılmaz F, Tunç O, Isayev A. Access cavity preparation and localization of root canals using guides in 3D-printed teeth with calcified root canals: An in vitro CBCT study. Diagnostics. 2023;13:2215.
10. Vinagre A, Castanheira C, Messias A, Palma PJ, Ramos JC. Management of pulp canal obliteration—systematic review of case reports. Medicina. 2021;57(11):1237.
11. Mahjourian Qomi R, Aminsobhani M, Assadian H, Adnaninia ST. Innovative endodontic management of pulp canal obliteration in mandibular incisors using a static navigation system: A case report. Clin Case Rep. 2024;12(11):e9596.
12. Gürsoy Emek B, Kurnaz S, Kiraz G, Kaya Mumcu A. Use of three-dimensional endodontic guide in a maxillary premolar tooth with an obliterated root canal: A case report. Turk Endod J. 2022;7(2):79-84.
13. Cruci P, Zavattini A, Gaon A, Patel M, Al Salehi SK. A grading system for pulp canal calcification based upon 3-D imaging to aid in assessment of case difficulty in orthograde endodontic treatment. J Endod. 2025;S0099-2399(25)00470-4.

14. Zahran SS, Alamoudi RA. Radiographic evaluation of teeth with pulp stones and pulp canal obliteration: characteristics and associations with dental parameters. Libyan J Med. 2024;19(1).
15. Kumar A, Ravisankar M, Paulaian B, Meyappan N. Endodontic management of calcified maxillary central incisors using CBCT: A report of two cases. J Oper Dent Endod. 2022;6(2):74-77.
16. Jiandong B, Yunxiao Z, Zuhua W. Generalized pulp canal obliteration in a patient on long-term glucocorticoids: A case report and literature review. BMC Oral Health. 2022;22:352.
17. Nabavi S, Latifian E. Guided endodontic technique in mandibular incisors with pulp canal obliteration: A case report. Iran Endod J. 2025;20(1):e21.
18. Zargar N, Amiri M. Guided endodontic treatment for calcified central incisor with discoloration, a conservative approach preserving incisal edge: A case report. Iran Endod J. 2023;18(4):259-263.
19. Doranala S, Vemisetty H, Punna R, Alwala AM. Endodontic management of canal calcification in maxillary central incisor using 3D prototyping technique: A case report. J Adv Oral Res. 2020;11(1):93-96.
20. Boquete-Castro A, Lopez AP, Martins AS, Lorenzo AS, Perez PR. Applications and advantages of the use of cone-beam computed tomography in endodontics: An updated literature review. Saudi Endod J. 2022;12(2):168-174.
21. Kazimierczak W, Kazimierczak N, Issa J, Wajer R, Wajer A, Kalka S, Serafin Z. Endodontic treatment outcomes in cone beam computed tomography images—assessment of the diagnostic accuracy of AI. J Clin Med. 2024;13(14):4116.
22. Patel S, Brown J, Semper M, Abella F, Mannocci F. European Society of Endodontology position statement: Use of cone beam computed tomography in Endodontics: European Society of Endodontology (ESE) developed by. Int Endod J. 2019;52(12):1675-1678.
23. Mohammed H. Clinical use of CBCT in endodontics: a case series following ESE position statement. *Ann Stomatol (Roma).* 2025;16(2):75–86.

INFORMATION FOR AUTHORS

AIMS AND SCOPE

Experimental and Applied Biomedical Research (*EABR)* former *Serbian Journal of Experimental and Clinical Research* is a peer-reviewed, open access journal which publishes original research articles, reviews, case reports and letters to the editor in all areas of the biomedical sciences that have not been published previously. The journal comprises both basic and clinical research in the field of biomedicine. Current acceptance rate is 60%. *EABR* was founded in 2000 under the name *Medicus* and over more than two decades has grown into one of the leading national journals in the field of biomedical sciences. *Experimental and Applied Biomedical Research* is owned and published by Faculty of Medical Sciences University of Kragujevac. The journal adheres to the policies of the International Committee of Medical Journal Editors (ICMJE) and publishing ethics guidelines provided by the Committee on Publication Ethics (COPE).

TYPES OF MANUSCRIPTS

- *Original research articles*: *EABR* considers all original research manuscripts which present the results of an original research study (experimental or clinical). These manuscripts must contain sufficient information on all relevant research methods, as well as a detailed analysis of the results obtained.

- *Reviews*: *EABR* considers literature reviews, systematic reviews and meta analyses addressed to a particular subject area, with special reference to new knowledge and facts. Manuscripts in this category must not be shorter than 6000 words, the text must cite more than 70 references of which 50% have been published in the previous 5 years. Systematic reviews should follow the PRISMA guidelines.

- *Case reports*: *EABR* considers case reports presenting detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports should usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches. Case reports should follow the CARE guidelines.

- *Letters to the editor: EABR* considers letters to the editor related to different clinico-laboratory observations. They should be titled, not exceed 500 words, and have a maximum of 5 references. Up to 1 table or figure may be submitted, but will be published at the discretion of the Editor. No more than 3 authors should appear.

MANUSCRIPT SUBMISSION

Manuscripts submitted to *Experimental and Applied Biomedical Research* must neither be published previously nor be under consideration for publication in another journal. Manuscripts are acompanied with a suitable *cover letter* stating that: the manuscript is not

submitted for publication elsewhere; all authors have agreed to submission; the study is carried out in accordance with relevant ethical international guidelines.

EABR considers only manuscripts written in English using *Microsoft Office Word* format and uploaded online at https://www.editorialmanager.com/sjecr/.

Plagiarism, data fabrication and image manipulation are not tolerated. Plagiarism includes copying text, ideas, images, or data from another source, even from authors own publications, without providing any reference to the original source. If a study's design or the manuscript's structure or language has been inspired by previous works, these papers must be explicitly cited. All manuscripts submitted to *Experimental and Applied Biomedical Research* are checked for plagiarism using the academic standard software prior to the first step of the editorial process.

MANUSCRIPT PREPARATION AND ORGANISATION

Title Page

The Title Page should contain the following informations:

- Manuscript title
- Full author(s) names
- The affiliation(s) of the author(s)
- A clear indication and an active e-mail address of the corresponding author

Manuscript title should be concise and informative.

It is necessary to state the full names and surnames (middle letter or name is optional) of all authors and the exact affiliations of all authors - institution, (department), city, (state), country. *Experimental and Applied Biomedical Research* remains neutral with regard to jurisdictional claims in institutional affiliations. Responsibility for affiliations ultimately rests with the author.

Abstract

Provide an abstract of 150 to 250 words. Abstract should be structured (Background, Methods, Results, Conclusion), citation-free, without abbreviations if possible.

Keywords

Three to five relevant keywords need to be added after the abstract. Keywords should be specific to the manuscript, yet reasonably common within the subject discipline.

Text Formatting

Manuscripts should be submitted in *Microsoft Office Word*. The authors should use normal, plain *Times New Roman* font (12pt) for text. Pages should be numbered automatically. Italics may be used for emphasis. Abbreviations should be defined at the first mentioning in the text and used consistently thereafter (do not use a separate subtitle for abbreviations only). Please use no more than three levels of displayed headings. International System (SI) of Units should be used (imperial, US customary and other units should be converted to SI units).

Original research articles should contain following sections: Introduction, Materials and Methods, Results, Discussion, Conclusions, Acknowledgments, Conflict of Interest, and References. *Reviews* may require different formats, while *Case reports* manuscripts should follow the CARE guidelines.

Introduction. This section should contain context or background for the study, rationale, clear aim of research or tested hypothesis.

Materials and Methods. This section should provide sufficient detail for replication of the study. If more than one method is used in the research, use subsections with appropriate subheadings. The *Materials and Methods* section should also contain following statements:

a) Informed Consent Statement. In cases where the identification of personal information is necessary for scientific reasons, authors should obtain informed consent from all individuals included in the study
b) Human Right Statement. Manuscripts containing information related to human should clearly state that the research has complied with all relevant international and national regulations and institutional policies and has been approved by the authors' institutional Ethics committee.
c) Animal Right Statement. Manuscripts containing information related to animals should clearly state that the research has complied with all relevant international and national regulations and institutional policies and has been approved by the authors' institutional Ethics committee.

For details and examples of statements please see part 'Research and publication ethics'.

Results. The results should be presented in logical sequence in the manuscript. Do not repeat all the data in the tables or figures in the text.

Conclusions. Within the *Conclusions* section the authors should clearly explain the main conclusions of the article, highlighting its importance and relevance.

Acknowledgments. Acknowledgments of people, grants, funds, etc. should be placed in a separate section after the Conclusions section. The names of funding organizations should be written in full. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Conflict of Interest. Authors must declare all relevant interests that could be perceived as conflicting. If there is no conflicts exist, the authors should state this. Submitting authors are responsible for coauthors declaring their interests.

References. *References* must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. In the text, reference numbers should be placed in round brackets ( ), and placed before the punctuation – e.g. (1), (1–3) or (1, 3). The list of references should only include works that are cited in the text and that have been published or accepted for publication. Personal communications and unpublished works should only be mentioned in the text.

The reference list should include contain surnames and the first letter of the author's name, full title, abbreviated title of the journal, year of publication, volume, number and pagination (Vancouver style guide). In case where the list of authors are more than six, please use et al. after the sixth author.

The examples of correct referencing:

For journal papers:
Shoji F, Haro A, Yoshida T, Ito K, Morodomi Y, Yano T, et al. Prognostic significance of intratumoral blood vessel in- vasion in pathologic stage IA non-small cell lung cancer. Ann Thorac Surg. 2010;89(3):864-9.

For journal papers by DOI:
Ewy MW, Patel A, Abdelmagid MG, Mohamed Elfadil O, Bonnes SL, Salonen BR, et al. Plant-Based Diet: Is It as Good as an Animal-Based Diet When It Comes to Protein? Curr Nutr Rep. 2022. doi: 10.1007/s13668-022-00401-8.

For books:
Kleiner FS, Mamiya CJ, Tansey RG. 2001. Gardner's art through the ages (11th ed.). Fort Worth, USA: Harcourt College Publishers.

For chapter in an edited book:
Mettam GR, Adams LB. How to prepare an electronic version of your article. In: Jones BS, Smith RZ, editors. Introduction to the electronic age, New York: E-Publishing Inc; 2009, p. 281–304.

Tables, figures and images

Tables

Tables should always be cited in text in consecutive numerical order. For each table, please supply a table caption (title) explaining the components of the table. Identify any previously published material by giving the original source in the form of a reference at the end of the table caption. Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

Figures

Please submit each figure as an individual file separate from the manuscript text. All figures are to be numbered using Arabic numerals. Figures should always be cited in text in consecutive numerical order. Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.

For vector graphics, the preferred format is EPS, for halftones, please use TIFF format. *Microsoft Office* files are also acceptable. Vector graphics containing fonts must have the fonts embedded in the files.

Line art:

- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

Halftone art:

- Definition: Photographs, drawings, or paintings with fine shading, etc.
- If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
- Halftones should have a minimum resolution of 300 dpi.

Images

Supply vector-based files such as those produced by *CorelDraw*, *Adobe Illustrator* or similar software. Vector files give us maximum flexibility for sizing your figures properly. Do not rasterize line art or text. Photographic images should have a minimum resolution of 300 dpi at final print size. Embedded images within a vector file should also have a minimum resolution of 300 dpi. Up sampling artwork (artificially increasing file size or resolution) will not improve quality and causes production problems. At final print size, line weights can be no thinner than 0.28 pt.

PEER REVIEW PROCESS

All submitted manuscripts received by the Editorial Office will be evaluated by a professional *Editorial board* to determine whether they posses sufficient quality, are they properly prepared and follow the ethical policies of *Experimental and Applied Biomedical Research*. Manuscripts that do not fit with the quality and ethical standards of *EABR* will be rejected before peer-review. Manuscripts that are not properly prepared according to the Instruction for authors will be returned to the authors for revision and resubmission.

Once a manuscript passes the initial evaluation, it will be assigned to at least two independent experts for single-blind peer-review process. If the outcomes of the performed reviews are opposite, the third review is required. The peer-review outcomes are one of following:

- *Accept (without any changes)* - the journal will publish the paper in its original form. This type of decision outcome is rare.
- *Minor revision* - the manuscript have high chance to be accepted after fulfillment of minor corrections. Authors will be asked to resubmit the revised manuscript within a suitable time frame, and the revised version will be returned to the reviewer for further comments.
- *Reconsider after Major Revision* - the acceptance of the manuscript would depend on the revisions. The authors are required to perform extensive and significant

improvements in their manuscript. Authors will be asked to resubmit the revised manuscript within a suitable time frame, and the revised version will be returned to the reviewer for further comments.

- *Reject* - the manuscript is rejected for two reasons: 1. it has serious flaws, and/or makes no original significant contribution; 2. corrections and improvements during the (major) revision were not sufficient and satisfactory. No offer of resubmission to the journal is provided.

All reviewer comments should be responded point-by-point in a separate document entitled 'Answers to reviewers comments'. Corrections should be marked within the text in a red colour or as a track changes. During the submission process, author should suggest two potential reviewers with the appropriate expertise to review the manuscript. Proposed reviewers should be from different institutions than the authors.

Upon editor's approval, after received positive manuscript reviews, the manuscript is accepted in the system, and the corresponding author receives information about the manuscript accepted for publication to the email address. Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, pagination and publication. The Editorial board reserves the right to correct the English language after proofreading by the authors.

DOI number is assigned to the paper and, after proofreading and text break according to the Journal instructions, the paper is published as *Ahead of Print* first on *Sciendo* platform (https://sciendo.com/journal/sjecr) and then in one of the next issues of the Journal.

RESEARCH AND PUBLICATION ETHICS

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigation was carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013. As a minimum, a statement including number of approval and name of the ethics committee must be stated in Section 'Statement of Human Rights' of the article. In addition, the protection of privacy is a legal right that must not be breached without individual informed consent. In cases where the identification of personal information is necessary for scientific reasons, authors should obtain full documentation of informed consent, including written permission from the patient prior to inclusion in the study.

Example of Statement of Human Rights: "The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of *Name of the Institution* (No. *number of approval*)."

Example of Statement of Informed Consent: "All subjects gave their informed consent for inclusion before they participated in the study".

For non-interventional studies (e.g. surveys, questionnaires, social media research), all participants must be fully informed if the anonymity is assured, why the research is being conducted, how their data will be used and if there are any risks associated. As with all

research involving humans, ethical approval from an appropriate ethics committee must be obtained prior to conducting the study.

Research involving Animals

When reporting on research that involves animal subjects, animal material or animal tissues, authors must declare that the investigation was carried out following the rules of the European Directive for the welfare of laboratory animals (No. 2010/63/EU) and national and institutional regulations. As a minimum, a statement including number of approval and name of the ethics committee must be stated in Section 'Statement of Animal Rights' of the article. Statements on animal welfare should confirm that the study complied with all relevant legislation. Also, authors must include details on housing, husbandry and pain management in their manuscript (section Materials and methods).

Example of Statement of Animal Rights: "All research procedures were carried out in strict accordance with the European Union Directive for the welfare of laboratory animals (No. 2010/63/EU) and approved by the Ethics Committee of *Name of the Institution* (No. *number of approval*)."

EABR